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ON THE CHANGE OF POPULATION FITNESS BY NATURAL SELECTION * †

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1. INTRODUCTION

In *The Origin of Species*, Darwin (1859) put forward the view that natural selection, by preserving and accumulating small inherited modifications advantageous to each organism, is continuously effecting the improvement of the species in relation to its organic and inorganic environment. At that time the mechanism of particulate inheritance was unknown and he could not reject the theory of blending inheritance nor even the inheritance of acquired characters. Nevertheless it is remarkable that he did arrive at the right conclusion on the role of natural selection in evolution.

Later, based on the theory of Mendelian inheritance, Fisher (1930) formulated his " fundamental theorem of natural selection " by equating the rate of increase of population fitness with the genetic variance in fitness. Since the variance is a non-negative quantity, fitness in this formulation always increases with time. This view seems to be compatible with the general picture of evolution which has taken place in the past and may be considered as a quantitative description of an essential feature of evolution. In fact, Fisher (1930) claims that this law should hold the same position among the biological sciences as the second law of thermodynamics in physical sciences. However, if one considers actual cases in which competition with other species or deterioration of environment are involved, additional terms may be necessary and Fisher (1930) derived an equation for the rate of increase of the Malthusian parameter of the population in which the effect of overpopulation (C), genetic variance (W) and the effect of deterioration of environment (D) are included.

The validity of the " fundamental theorem " for the case of variable selection coefficients has been questioned by Wright (1949) who later derived an additional term for such a case (Wright, 1950). Attempts to obtain more general expressions for the rate of increase of population fitness have been made by Crow and Kimura (1956), Wright (1955) and Crow (1955).

The present paper deals with four problems : (1) I shall give a general equation for the role of additive, dominance, and epistatic components of fitness in determining the rate of change in population fitness. No restriction is placed on the mating system. A few of the many interesting special cases are discussed. (2) The process of

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natural selection will be treated from a different view, and a new formulation given which may be called a " maximum principle in the genetic theory of natural selection ". (3) A short section is devoted to the change in population variability with selection. (4) Finally these results will be applied to artificial selection, with special reference to the roles of dominance and epistatic components when inbreeding and linkage are involved.

2. MEASUREMENT OF FITNESS WITH TWO MODELS OF GENERATION TIME

Before going into the detailed argument on the main subject, it might be preferable to explain the mathematical models used and to define " fitness " for each model.

2.1. *A model with a discrete time parameter*

In this model, all the members of the population at a given moment belong to the same generation. After each breeding cycle the population is completely replaced by its progeny to form the next generation. Thus the generation time, t, takes only integral values ; 0, 1, 2, etc. The fitness of each genotype is measured by the average number of offspring per individual. The letter w will be used to designate fitness in this sense and following Wright (1942 and later) will be called the selective value of the genotype. The population is stationary in size if the mean fitness ($\bar{w}$) is unity. The selection coefficient of the genotype A_iA_j denoted by s_{ij} will be defined by

$$s_{ij} = w_{ij} - 1,$$

where w_{ij} is the selective value of A_iA_j.

Quite often, an arbitrary value of unity is assigned to one of the genotypes, but this is not meaningful unless the aim of study is restricted to the change of gene frequencies.

Consider an arbitrary number of alleles at one locus and let x_i be the relative frequency of the i^{th} allele A_i. Under this model, the rate of change of the gene frequency per generation, Δx_i, is given by

$$\Delta x_i = \frac{x_i(w_{i\cdot} - \bar{w})}{\bar{w}}, \tag{2.1.1}$$

where $w_{i\cdot} = \sum_j w_{ij}P_{ij}/x_i$ and $\bar{w} = \sum_{ij} w_{ij}P_{ij} = \sum_i w_{i\cdot}x_i$. P denotes the zygotic frequency such that P_{ii} is the frequency of the homozygote A_iA_i while $2P_{ij}(i \neq j)$ is the frequency of the heterozygote $A_iA_j(P_{ij} = P_{ji})$. The definition of P is complicated for the multilocus case, but the difficulty may be avoided by saying that P_{ij} (for arbitrary i and j) stands for the frequency of the *ordered genotype* A_iA_j, i.e. for mathematical convenience we distinguish between A_iA_j and A_jA_i.

2.2. *A model with a continuous time parameter*

In this model a population may contain individuals of different ages as in human populations. For a rigorous treatment of such a problem integral equations are needed (Haldane, 1927).

An appropriate scale for measuring fitness is the Malthusian parameter, m (Fisher, 1930), which measures the rate of population increase or decrease. It is defined as the real root of the equation ;

$$\int_0^\infty e^{-mx} l_x b_x dx = 1,$$

where l_x is the proportion of the population which survive to age x, and b_x is the rate of reproduction at that age. To obtain the fitness, m_{ij}, of the genotype A_iA_j, the above equation may be applied to a group of individuals of genotype A_iA_j, though perhaps differing at other loci. If there are differences in the vital statistics of the two sexes, " we may, certainly in mankind, and with a strong presumption in all other species, effectively average out the sex differences " (Fisher, 1953).

In the present paper, I will designate the fitness measured in Malthusian parameters by the letter a such that a_{ij} is the fitness of A_iA_j. Let n_{ij} be the number of individuals with the ordered genotype A_iA_j in the population. Since a_{ij} represents the net reproductive rate of the genotype A_iA_j such that the contribution to the rate of increase of the population number N from A_iA_j individuals is $n_{ij}a_{ij}$, then if N_i is the number of A_i genes such that

$$x_i = N_i/N,$$

we have

$$\frac{dN_i}{dt} = \sum_j n_{ij}a_{ij} = N\sum_j P_{ij}a_{ij}. \tag{2.2.1}$$

Therefore

$$\frac{dN}{dt} = N \sum_{ij} P_{ij}a_{ij}$$

and we obtain

$$\frac{dx_i}{dt} = \frac{d}{dt}\left(\frac{N_i}{N}\right) = \frac{1}{N}\frac{dN_i}{dt} - \frac{N_i}{N^2}\frac{dN}{dt}$$

$$= \sum_j P_{ij}a_{ij} - x_i \sum_{ij} P_{ij}a_{ij}$$

or more simply

$$\frac{dx_i}{dt} = x_i(a_{i\cdot} - \bar{a}), \tag{2.2.2}$$

where $a_{i\cdot} = \sum_i a_{ij}P_{ij}/x_i$ and $\bar{a} = \sum_{ij} a_{ij}P_{ij}$. The quantity $a_{i\cdot} - \bar{a}$ will be called the average excess of the gene A_i and will be designated by $\tilde{a}_{i\cdot}$. Throughout this paper a bar will be used to designate the population mean while a tilde will designate the deviation from the mean. Thus

(2.2.2) is equivalent to saying that the logarithmic rate of increase of gene frequency is equal to the average excess of the gene in fitness, *i.e.*

$$\hat{x}_i = \bar{a}_{i\cdot}, \tag{2.2.3}$$

in which

$$\hat{x}_i = \frac{d}{dt} \log x_i.$$

2.3. *Interrelation between the two models*

The above two models are both abstractions from reality and each has its advantages and disadvantages. For example, the discrete model might be suitable for an annual plant population while the continuous model would be preferable for the treatment of a human population. Though the scales of fitness for the two models are different, there are certain correspondences between them. Roughly speaking, m_{ij} or a_{ij} is equivalent to $\log w_{ij}$. A formal passage from (2.1.1) to (2.2.2) is attained as follows. Write $w_{ij} = 1 + s_{ij}\Delta t$ and substitute in (2.1.1) to give

$$\frac{\Delta x_i}{\Delta t} = \frac{x_i(s_{i\cdot} - \bar{s})}{1 + \bar{s}\Delta t}.$$

By letting $\Delta t \to 0$, we have (2.2.2) except that s_{ij} appears instead of a_{ij}. This suggests a formal correspondence between s_{ij} in the discrete model and a_{ij} in the continuous model.

Mathematically, the discrete model requires the calculus of finite differences, while the continuous model permits application of the familiar differential calculus which often leads to a more elegant formulation. In the present paper I shall mainly employ the continuous model for this reason.

3. RATE OF CHANGE IN POPULATION FITNESS

We start by considering a single locus with an arbitrary number, say n, of alleles. No restriction will be imposed on the mating system. The fitness a_{ij} may be expressed in the following form,

$$a_{ij} = \bar{a} + \alpha_i + \alpha_j + d_{ij},$$

where $\bar{a}$ is the mean fitness of the population, α_i and α are respectively additive effects of the genes A_i and A_j on fitness and d_{ij} is the dominance deviation from linearity. Following Fisher (1930, 1941) we shall call α_i the average effect of the gene A_i. The α_i's are determined by minimising

$$\sum_{ij} d_{ij}^2 P_{ij}.$$

By writing $\bar{a}_{ij}$ for $a_{ij} - \bar{a}$, the quantity to be minimised is

$$Q = \sum_{ij} P_{ij}(\bar{a}_{ij} - \alpha_i - \alpha_j)^2.$$

Since $-\frac{1}{2}\frac{\partial Q}{\partial \alpha_k} = 0$ leads to

$$\sum_j P_{kj}(\bar{a}_{kj} - \alpha_k - \alpha_j) + \sum_i P_{ik}(\bar{a}_{ik} - \alpha_i - \alpha_k) = 0,$$

we have the normal equations for α_k's ;

$$2\alpha_k x_k + 2\sum_i P_{ki}\alpha_i = 2\sum_i P_{ki}\bar{a}_{ki}. \quad (k = 1, \ldots, n) \tag{3.1}$$

If we adopt the notation $\bar{a}_{k\cdot}$ for $\sum_i P_{ki}\bar{a}_{ki}/x_k$, the right side of (3.1) is written as $2x_k\bar{a}_{k\cdot}$ and the sum of squares due to the average effects is given by

$$V_g = 2\sum_k \alpha_k x_k \bar{a}_{k\cdot}. \tag{3.2}$$

If we note (2.2.2) this may also be written as

$$V_g = 2\sum_i \alpha_i \frac{dx_i}{dt}. \tag{3.2}'$$

It may be convenient to note for later discussion that under random mating α_i is equal to $\bar{a}_{i\cdot}$ and

$$V_g = 2\sum_i x_i \bar{a}_{i\cdot}^2. \tag{3.2}''$$

For the derivation of normal equations (3.1) and the extraction of the sum of squares due to α_i's, the reader may refer to Kempthorne (1957).

We will call V_g the *genic* variance in fitness ; it has also been called the additive genetic variance in fitness. Fisher (1930) called the same quantity the genetic variance.

Now we introduce a new quantity θ_{ij} defined by

$$\theta_{ij} = \frac{P_{ij}}{x_i x_j}. \quad (\theta_{ij} = \theta_{ji}). \tag{3.3}$$

θ_{ij} may be called the coefficient of departure from random combination of alleles and is unity if mating is random. Substituting $P_{ki} = x_k x_i \theta_{ki}$ in (3.1) and dividing both sides of the equation by $2x_k$, we have

$$\alpha_k + \sum_i x_i\theta_{ki}\alpha_i = \bar{a}_{k\cdot} = \sum_i x_i\theta_{ki}\bar{a}_{ki},$$

or

$$\sum_j x_j\theta_{ij}\bar{a}_{ij} = \alpha_i + \sum_j \alpha_j\theta_{ij}x_j. \tag{3.4}$$

Let us now consider the rate of increase in population fitness under natural selection. Since

$$\bar{a} = \sum_{ij} a_{ij}P_{ij},$$

we have

$$\frac{d\bar{a}}{dt} = \sum_{ij}\frac{da_{ij}}{dt}P_{ij} + \sum_{ij} a_{ij}\frac{dP_{ij}}{dt},$$

or more simply, employing the Newtonian method of denoting a time derivative by a superior dot,

$$\dot{\bar{a}} = \sum_{ij}\dot{a}_{ij}P_{ij} + \sum_{ij} a_{ij}\dot{P}_{ij}. \tag{3.5}$$

The first term in the right side is the population average of the rate of change of individual fitness and may be denoted by

$$\overline{\frac{da}{dt}} \text{ or } \bar{\dot{a}}. \tag{3.6}$$

The second term on the right is

$$\sum_{ij} a_{ij}\dot{P}_{ij} = \sum_{ij} (\bar{a}+\tilde{a}_{ij})\dot{P}_{ij} = \bar{a}\sum_{ij}\dot{P}_{ij} + \sum_{ij}\tilde{a}_{ij}\dot{P}_{ij},$$

and since $\sum_{ij} P_{ij} = 1$ or $\sum_{ij}\dot{P}_{ij} = 0$, it reduces to

$$\sum_{ij}\tilde{a}_{ij}\dot{P}_{ij},$$

which upon substitution of $P_{ij} = x_i x_j \theta_{ij}$ becomes

$$\sum_{ij}\tilde{a}_{ij}\dot{x}_i x_j\theta_{ij} + \sum_{ij}\tilde{a}_{ij}x_i\dot{x}_j\theta_{ij} + \sum_{ij}\tilde{a}_{ij}x_i x_j\dot{\theta}_{ij}. \tag{3.7}$$

If we substitute (3.4) in the first term of the above expression, we have

$$\sum_i \dot{x}_i(\alpha_i + \sum_j \alpha_j\theta_{ij}x_j) = \sum_i \alpha_i\dot{x}_i + \sum_{ij}\alpha_j\dot{x}_i x_j\theta_{ij}. \tag{3.8}$$

It will be immediately seen from (3.2)′ that the first term of the last expression is

$$\tfrac{1}{2}V_g.$$

By definition $\sum_i P_{ij} = x_j$ or $\sum_i x_i x_j\theta_{ij} = x_j$, and we get $\sum_i x_i\theta_{ij} = 1$ or differentiating both sides we get $\sum_i \dot{x}_i\theta_{ij} + \sum_i x_i\dot{\theta}_{ij} = 0$. Thus the second term of (3.8) reduces to

$$\sum_j \alpha_j x_j(-\sum_i x_i\dot{\theta}_{ij}) = -\sum_{ij}\alpha_j x_i x_j\dot{\theta}_{ij}.$$

Similarly the second term of (3.7) becomes

$$\tfrac{1}{2}V_g - \sum_{ij}\alpha_i x_i x_j\dot{\theta}_{ij}.$$

Therefore (3.7) can be written in the form

$$V_g + \sum_{ij}(\tilde{a}_{ij} - \alpha_i - \alpha_j)x_i x_j\dot{\theta}_{ij},$$

or

$$V_g + \sum_{ij} d_{ij}P_{ij}(\dot{\theta}_{ij}/\theta_{ij}).$$

Thus the required expression for the rate of change of population fitness by selection is

$$\frac{d\bar{a}}{dt} = V_g + \overline{\frac{da}{dt}} + \sum_{ij} P_{ij}d_{ij}\frac{d}{dt}\log\theta_{ij} \tag{3.9}$$

or in more simple notation

$$\dot{\bar{a}} = V_g + \bar{\dot{a}} + \overline{d\cdot\dot{\theta}}, \tag{3.10}$$

in which the open circle over θ denotes the time derivative of $\log \theta$ as in (2.2.3). Also it is convenient to note that

$$\sum_{ij} P_{ij} d_{ij} \frac{d}{dt} \log (P_{ij}/x_i x_j) = \sum_{ij} P_{ij} d_{ij} \frac{d}{dt} \log P_{ij} = \sum_{ij} d_{ij} \frac{d}{dt} P_{ij}.$$

Thus, as shown in (3.9) or (3.10), the rate of increase of the population fitness is decomposed into three components: (i) Genic variance in fitness. (ii) Average increase in the fitness of individual genotypes. This term is zero if genotypic fitnesses remain constant. (iii) A contribution due to dominance deviations and mating system. This term vanishes under random mating ($\theta_{ij} = 1$), or under any system of mating if there is no dominance. It is interesting to note the simple relation by which dominance components appear in the expression. Each component is weighted by the rate of change of the logarithm of the corresponding coefficient of departure from random combination of alleles. This would suggest a similar relation for epistatic components when two or more loci are involved.

Without going into the details of derivation, the result for the two loci may be summarised as follows. We decompose the fitness of the genotype $A_i A_j B_k B_l$ in the form

$$a_{ijkl} = \bar{a} + A_{ij} + B_{kl} + \epsilon_{ijkl},$$

where A_{ij} is the additive effect of the first locus (a), B_{kl} that of the second locus (b) and ϵ_{ijkl} is the epistatic deviation. Let

$$A_{ij} = \alpha_i + \alpha_j + d_{ij}^{(a)}$$

and

$$B_{kl} = \beta_k + \beta_l + d_{kl}^{(b)}$$

in which α and β stand for the additive effects of the alleles for the first and second loci respectively and $d^{(a)}$ and $d^{(b)}$ are dominance deviations for the two loci. Let P_{ijkl} be the frequency of the ordered genotype $A_i A_j B_k B_l$ and let $P_{ij}^{(a)}$ be the frequency of the ordered genotype $A_i A_j$ in which no attention is paid to the second locus, i.e. $P_{ij}^{(a)} = \sum_{kl} P_{ijkl}$, and $P_{kl}^{(b)}$ be the similar frequency of the ordered genotype $B_k B_l$ at the second locus. If we designate the frequencies of genes A_i and B_k by x_i and y_k respectively, we can define coefficients of departure from random combination of alleles at the two loci by

$$\theta_{ij}^{(a)} = \frac{P_{ij}^{(a)}}{x_i x_j} \text{ and } \theta_{kl}^{(b)} = \frac{P_{kl}^{(b)}}{y_k y_l},$$

respectively. Similarly we introduce the coefficient of departure involving two loci by

$$\theta_{ijkl} = \frac{P_{ijkl}}{P_{ij}^{(a)} P_{kl}^{(b)}}.$$

Then

$$\frac{d\bar{a}}{dt} = V_g + \overline{\frac{da}{dt}} + \sum_{ij} P_{ij}^{(a)} d_{ij}^{(a)} \frac{d}{dt} \log \theta_{ij}^{(a)}$$
$$+ \sum_{kl} P_{kl}^{(b)} d_{kl}^{(b)} \frac{d}{dt} \log \theta_{kl}^{(b)} + \sum_{ijkl} P_{ijkl} \epsilon_{ijkl} \frac{d}{dt} \log \theta_{ijkl},$$

or more simply

$$\dot{\bar{a}} = V_g + \overline{\dot{a}} + \overline{\delta_{ij}^{(a)} d_{ij}^{(a)}} + \overline{\delta_{kl}^{(b)} d_{kl}^{(b)}} + \overline{\delta_{ijkl} \epsilon_{ijkl}}. \tag{3.11}$$

The epistatic effect may further be subdivided into additive × additive, additive × dominance and dominance × dominance effects. To simplify the following formulation we adopt a rule that the letters i, j, k and l refer respectively to the left gene in the first locus, the right gene in the first locus, the left gene in the second locus and the right gene in the second locus in the ordered genotype $A_iA_jB_kB_l$. With this abbreviation, not only subscripts a and b are unnecessary but also simple expressions like P_{ik}, P_{ijk}, ..., etc. may be used for the frequencies of ordered genotypes A_i-B_k-, $A_iA_jB_k$-, etc.

Let
$$\epsilon_{ijkl} = (\alpha\beta)_{ik} + (\alpha\beta)_{il} + (\alpha d)_{ikl}$$
$$+ (\alpha\beta)_{jk} + (\alpha\beta)_{jl} + (\alpha d)_{jkl}$$
$$+ (d\beta)_{ijk} + (d\beta)_{ijl} + (dd)_{ijkl}.$$

Corresponding to each of the dominance and epistatic components, coefficients of departure from random combination are defined in the obvious way :

$$\theta_{ij} = P_{ij}/x_ix_j,\ \theta_{ik} = P_{ik}/x_iy_k, \ldots,$$
$$\theta_{ijk} = P_{ijk}/P_{ij}y_k,\ \theta_{ijkl} = P_{ijkl}/P_{ij}P_{kl}.$$

Then

$$\dot{\bar{a}} = V_g + \overline{\dot{a}} + \overline{\delta_{ij} d_{ij}} + \overline{\delta_{kl} d_{kl}}$$
$$+ \overline{\delta_{ik}(\alpha\beta)_{ik}} + \ldots + \overline{\delta_{ijkl}(dd)_{ijkl}}. \tag{3.12}$$

The law of formulation of each term is self-evident so no further explanation is necessary.

Thus for an arbitrary number of loci each with an arbitrary number of alleles, we can write the rate of increase in population fitness as follows :

$$\frac{d\bar{a}}{dt} = V_g + \overline{\frac{da}{dt}} + \overline{\Sigma \epsilon \frac{d}{dt} \log \theta}, \tag{3.13}$$

in which ϵ stands for an interaction effect (dominance or epistatic) and θ is the corresponding coefficient of departure from random combination. The summation extends over all possible intra- and inter-locus interactions.

4. SOME SPECIAL SITUATIONS

4.1. For constant genotypic fitnesses, $\dot{\bar{a}} = 0$ and (3.10) becomes

$$\dot{\bar{a}} = V_g + \overline{d \cdot \dot{\theta}}.$$

If there exists a constant λ_{ij} such that

$$\lambda_{ij} = P_{ij}^2 / P_{ii} P_{jj}$$

for all i and j, then $\dot{\bar{a}} = V_g$. This is easily shown as follows :

Since $P_{ij} = x_i x_j \theta_{ij}$, we have $\lambda_{ij} \theta_{ii} \theta_{jj} = \theta_{ij}^2$, or

$$\log \lambda_{ij} + \log \theta_{ii} + \log \theta_{jj} = 2 \log \theta_{ij}.$$

Differentiating both sides with respect to time,

$$\dot{\theta}_{ii} + \dot{\theta}_{jj} = 2\dot{\theta}_{ij}.$$

Thus
$$\overline{d \cdot \dot{\theta}} = \sum_{ij} d_{ij} \dot{\theta}_{ij} P_{ij} = \tfrac{1}{2} \sum_{ij} d_{ij} (\dot{\theta}_{ii} + \dot{\theta}_{jj}) P_{ij}$$

$$= \tfrac{1}{2} \sum_i \dot{\theta}_{ii} \sum_j d_{ij} P_{ij} + \tfrac{1}{2} \sum_j \dot{\theta}_{jj} \sum_i d_{ij} P_{ij} = 0,$$

because
$$\sum_j d_{ij} P_{ij} = \sum_i d_{ij} P_{ij} = 0.$$

The equivalent result was obtained by Kempthorne (1957) by using matrix algebra. However, Fisher (1941) had already demonstrated such a result for the two allelic case. Extension to the multi-locus case is immediately apparent from (3.13).

In the case of inbreeding in which zygotic frequencies are expressed by gene frequencies and the inbreeding coefficient F, $P_{ii} = Fx_i + (1-F)x_i^2$ for homozygotes and $P_{ij} = (1-F)x_i x_j$ for heterozygotes. Therefore $\theta_{ii} = \dfrac{1}{x_i} F + (1-F)$ and $\theta_{ij} = (1-F)$ $(i \neq j)$.

We will consider this case later in more detail.

4.2 A quantity analogous to the potential function in a conservative physical system may exist in some special cases. Wright (1955) considered such a quantity and termed it the internal selective value. I shall use a similar quantity which may be called the internal fitness (I) in the following way : We say that the internal fitness exists if

$$2 \sum_i \alpha_i dx_i \qquad (\sum_i x_i = 1)$$

is an exact differential with respect to gene frequencies $(x_1, x_2, \ldots, x_n)$. In that case there exists a function I such that the above expression is equated with dI. Therefore

$$\frac{dI}{dt} = 2 \sum_i \alpha_i \frac{dx_i}{dt} = V_g,$$

i.e. the rate of increase of internal fitness is equal to the genic variance

at that moment. Thus if the internal fitness is equal to the mean fitness of the population $\bar{a}$ except for an arbitrary constant, the fundamental theorem is satisfied. However, the reverse is not necessarily true and the derivation of Fisher's fundamental theorem does not presuppose or imply the existence of any such potential function.

4.3. When competition between genotypes is involved we sometimes encounter a rather peculiar situation. As an example let us consider three alleles A_1, A_2, and A_3 with frequencies x_1, x_2, and x_3. For simplicity we assume no dominance or more conveniently a haplont situation. Suppose an individual with gene A_1 has its fitness decreased by the amount c when it is surrounded by individuals with A_2, while A_2 if surrounded by the former A_1 gains fitness by the same amount

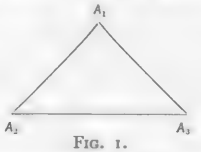

FIG. 1.

c. Similarly A_2 when surrounded by A_3 individuals loses fitness by c, while the reverse situation would add fitness c to an A_3 individual, and so on cyclically (fig. 1). Then the law of change of gene frequencies will be given by

$$(4.3.1)\qquad \left\{\begin{aligned} \frac{dx_1}{dt} &= cx_1(x_3-x_2) \\ \frac{dx_2}{dt} &= cx_2(x_1-x_3) \\ \frac{dx_3}{dt} &= cx_3(x_2-x_1). \end{aligned}\right.$$

The gene frequencies will move perpetually on a closed path * which is the intersection of a plane

$$x_1+x_2+x_3 = 1$$

and a surface

$$x_1x_2x_3 = C_0,$$

where C_0 is a constant determined by the initial conditions. In the present case $\alpha_1 = \tilde{a}_1 = c(x_3-x_2)$, $\alpha_2 = \tilde{a}_2 = c(x_1-x_3)$, $\alpha_3 = \tilde{a}_3 = c(x_2-x_1)$, and $\bar{a} \equiv 0$. This shows that, while gene frequencies change perpetually, no change in population fitness occurs :

$$\frac{d\bar{a}}{dt} = 0.$$

* With a discrete model, we have a set of finite difference equations $\Delta x_1 = cx_1(x_3-x_2)$, etc. instead of (4.3.1) and the path is not quite closed. In the neighbourhood of (1/3, 1/3, 1/3), the solution contains a factor $(1+c^2/3)^{1/2}$ showing that the path is an outwardly proceeding spiral. The author owes to Professor S. Wright and Mr Y. Hiraizumi for calling his attention to this fact.

Nevertheless the population at a given moment may contain plenty of genic variance, for

$$V_g = c^2\{x_1(x_3-x_2)^2+x_2(x_1-x_3)^2+x_3(x_2-x_1)^2\}.$$

The reason for this seeming contradiction is that the second term in our formula (3.9) always cancels out the first term, namely

$$\overline{\frac{da}{dt}} = -V_g,$$

so that

$$\frac{d\bar{a}}{dt} = V_g + \overline{\frac{da}{dt}}$$

still holds.

4.4 In our definition of fitness in (2.2.1), sexual difference in fitness for each genotype, if it exists, is in a sense averaged out in the expression. This is reasonable since under a complicated mating system it would not be practicable to analyse contributions due to different sexes. However, under simple circumstances this may be done to give interesting results. Let a_{ij} be the fitness of an A_iA_j female in producing offspring and let c_{ij} be the competitive ability of the A_iA_j male in fertilising females. We assume random mating and no dominance. Then we have

$$\frac{d\bar{a}}{dt} = \sigma_{aa}+\sigma_{ac}, \tag{4.4.1}$$

where σ_{aa} is half the genic variance,

$$\sigma_{aa} = \sum_i x_i(a_i.-\bar{a})^2$$

and σ_{ac} is the covariance between average excess (or effect in this case) of fitness in females and that of competitive ability in males.

$$\sigma_{ac} = \sum_i x_i(a_i.-\bar{a})(c_i.-\bar{c}),$$

in which $\bar{c}$ may be chosen to be zero. Let b be the regression coefficient of $c_i.$ on $a_i.$, i.e.

$$c_i. = \bar{c}+b(a_i.-\bar{a}),$$

where $b = \sigma_{ac}/\sigma_{aa}$. Then if b is less than -1,

$$\sigma_{ac} < -\sigma_{aa},$$

and therefore

$$\frac{d\bar{a}}{dt} = \sigma_{aa}+\sigma_{ac} < 0,$$

leading to the deterioration of the population. Yet the population may contain genic variance through this process.

$$V_g = 2\sigma_{aa} > 0.$$

In fact, Huxley (1942) put forward an explanation for the extinction

of species due to fixation of genes which enable males to be more successful in competition but make the species less fit to the external environment.

Fisher (1941) considered a hypothetical gene which enables the possessor to be self-fertilised but which has no effect on the seed productivity or cross-fertilising ability of the pollen. This is essentially equivalent to the case in which $\sigma_{aa} = 0$ in the above formulation. For, in this case $\sigma_{aa} = \sigma_{ac} = 0$ and

$$\frac{d\bar{a}}{dt} = 0,$$

which agrees well with what is expected from the fundamental theorem since

$$V_g = 2\sigma_{aa} = 0.$$

4.5. In a natural population there always exists an element of stochastic indeterminacy in the process of change in fitness. We consider here a simple single locus case with random mating.

Let δx_i be the rate of change in the frequency of A_i per unit time interval which may be taken as one generation, we can write

$$\delta x_i = x_i(a_i - \bar{a}) + \delta' x_i,$$

where $\delta' x_i$ is the change due to random sampling of gametes. Then the rate of change in population fitness is

$$\delta\bar{a} = \sum_{ij} \delta a_{ij} x_i x_j + V_g + 2 \sum_i a_i \delta' x_i.$$

Here a_{ij} is a random variable which may fluctuate from generation to generation. $\delta' x_i$'s are variables from multinomial distribution such that their mean, variance and covariance are given respectively by

$$M(\delta' x_i) = 0, \; V(\delta' x_i) = x_i(1 - x_i)/2N$$

and

$$\mathrm{Cov}(\delta' x_i \delta' x_j) = -x_i x_j / 2N \text{ for } i \neq j,$$

where N is the number of reproducing individuals in the population. Then the mean and variance of $\delta\bar{a}$ are

$$M(\delta\bar{a}) = \overline{M(\delta a)} + V_g, \tag{4.5.1}$$

and

$$V(\delta\bar{a}) = \overline{\sigma_{\delta a \delta a'}} + V_g/2N, \tag{4.5.2}$$

where

$$\overline{M(\delta a)} = \sum_{ij} M(\delta a_{ij}) x_i x_j$$

and

$$\overline{\sigma_{\delta a \delta a'}} = \sum_{iji'j'} \mathrm{Cov}(\delta a_{ij} \delta a_{i'j'}) x_i x_j x_{i'} x_{j'}.$$

The last term $V_g/(2N)$ corresponds to Fisher's error variance for his fundamental theorem (Fisher, 1930).

5. A MAXIMUM PRINCIPLE IN THE GENETICAL THEORY OF NATURAL SELECTION

So far we have considered how natural selection increases population fitness, but have paid little attention to how natural selection changes gene frequencies.

Let us start our consideration from a simple case with random mating and constant fitness of individual genotypes. Consider a locus with n alleles, say $A_1, A_2, \ldots, A_n$ with respective frequencies $x_1, x_2, \ldots, x_n$. First we will establish the following theorem : *For a given short time interval* δt, *natural selection causes gene frequency changes* $\delta x_1, \delta x_2, \ldots, \delta x_n$, *in such a way that the increase of population fitness,* $\delta\bar{a}$, *shall be maximum under the restriction*

$$\sum_{i=1}^{n} \frac{(\delta x_i)^2}{x_i} = \tfrac{1}{2} V_g (\delta t)^2. \tag{5.1}$$

To prove this theorem, we put $\delta\bar{a} = \eta$, $\delta x_i = \xi_i$ and $\frac{1}{2}V_g(\delta t)^2 = c$. Since

$$\bar{a} = \sum_{ij} a_{ij} x_i x_j,$$

we have, for any small set of gene frequency changes $(\xi_1, \xi_2, \ldots, \xi_n)$,

$$\delta\bar{a} = 2 \sum_{i=1}^{n} a_i . \delta x_i$$

or

$$\eta = 2 \sum_{i=1}^{n} a_i . \xi_i. \tag{5.2}$$

The restriction (5.1) is now written as

$$\sum_{1}^{n} \frac{\xi_i^2}{x_i} = c. \tag{5.1}'$$

In addition, since the sum of all the gene frequencies is unity, we inevitably have the obvious restriction :

$$\sum_{1}^{n} \xi_i = 0. \tag{5.3}$$

We are going to show that among all possible sets of gene frequency changes which satisfy (5.1)′ and (5.3), the one which is caused by natural selection is the set which makes η maximum.

For this purpose we introduce the Lagrange multipliers λ and μ and consider a function

$$\psi = \eta - \lambda\left(\sum_{1}^{n} \frac{\xi_i^2}{x_i} - c\right) - \mu\left(\sum_{1}^{n} \xi_i\right). \tag{5.4}$$

A set of ξ_i's which make ψ maximum may be obtained from

$$\frac{\partial \psi}{\partial \xi_i} = 2a_i. - 2\lambda \frac{\xi_i}{x_i} - \mu = 0,$$

or

$$2a_i.x_i - 2\lambda\xi_i - \mu x_i = 0. \tag{5.5}$$

Summing up both sides of (5.5) from $i = 1$ to n, and noting (5.3), we have

$$2\bar{a} = \mu.$$

Therefore (5.5) yields

$$\xi_i = (a_i. - \bar{a})x_i/\lambda. \tag{5.6}$$

By substituting this back into (5.1)′ we have

$$\sum_i x_i(a_i. - \bar{a})^2/\lambda^2 = c.$$

The denominator in the left side of the above equation is $\frac{1}{2}V_g$ from (3.2)″, while $c = \frac{1}{2}V_g(\delta t)^2$, so that

$$1/\lambda^2 = (\delta t)^2 \text{ or } 1/\lambda = \delta t,$$

because negative λ is inadequate. Thus (5.6) is

$$\xi_i = x_i(a_i. - \bar{a})\delta t$$

or

$$\frac{\delta x_i}{\delta t} = x_i(a_i. - \bar{a}), \quad (i = 1, \ldots, n)$$

which is equivalent to (2.2.2) and gives the law of change in gene frequencies due to natural selection.

Geometrical consideration may help in understanding the above theorem (fig. 2). We consider an $(n+1)$ dimensional space in which there are n coordinates for gene frequencies and one coordinate for population fitness. A population with a given set of gene frequencies is expressed as a point P in this space. A set of displacements ξ_i's together with a corresponding change in population fitness η define a vector radiating from P. Projection of this vector on the n dimensional subspace of gene frequencies is inevitably on the hyper plane given by (5.3). Then the theorem states that among all such vectors whose projections on such n dimensional subspace are on the hyper-ellipsoid given by (5.1)′, the vector corresponding to the change by natural selection is the one which gives maximum length of projection on the $\bar{a}$ axis.

The theorem can easily be extended to any number of loci. For example for two loci, we designate the displacement of gene frequencies in the second loci having m alleles by $\zeta_1, \zeta_2, \ldots, \zeta_m$, then (5.1)′ should be replaced by

$$\sum_1^n \frac{\xi_i^2}{x_i} + \sum^m \frac{\zeta_j^2}{} = \frac{1}{2}V_g(\delta t)^2 \tag{5.6}$$

and (5.3) by

$$\sum_1^n \xi_i = 0, \quad \sum_1^m \zeta_j = 0 \tag{5.7}$$

respectively.

The theorem may be further extended to cover a quite general situation in which the above restrictions of random mating and constant fitness of genotypes are removed. In what follows we will

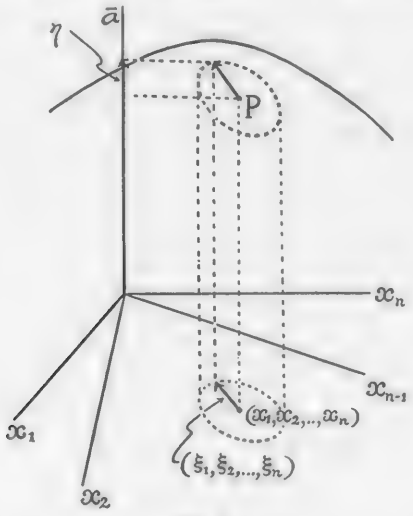

FIG. 2.

consider only the case of a single segregating locus, but the argument may easily be extended to any number of loci.

Let $$A = (\bar{a}_1., \bar{a}_2., \ldots, \bar{a}_n.)$$

be a vector whose components are average excesses of genes and let

$$D = (\xi_1, \xi_2, \ldots, \xi_n)$$

be a displacement vector in the set of gene frequencies. Then natural selection acts in such a way that the product

$$AD' = \sum_1^n \bar{a}_i.\xi_i$$

shall be maximum under the restriction

$$\sum_1^n \xi_i^2/x_i = \sigma_a^2(\delta t)^2,$$

where σ_a^2 is the variance of average excess in fitness per ploid, *i.e.*,

$$\sigma_a^2 = \sum_1^n x_i(a_i.-\bar{a})^2.$$

In this form the theorem is applicable not only to diploid but also to $2K$ ploid in general in which $K = \frac{1}{2}, 1, 2, 3, \ldots$, etc.

The above theorem is somewhat analogous to the principle of least action in physics and may be called the maximum principle in the genetical theory of natural selection. Whereas from the least action principle the laws of motion may be derived, we may from this theorem derive the rules of change in gene frequencies.

6. RATE OF CHANGE OF POPULATION VARIABILITY

Considering the great importance of the genic variance in evolution, it is appropriate to study how quickly the genic variance will be consumed by natural selection for the improvement of the species. Though the following result is quite restricted, it may help our understanding of the problem.

Consider a random mating population and assume constant fitness of genotypes. In this case the average effect is equal to the average excess and we have

$$V_g = 2\sum_{i=1}^{n} x_i(a_i - \bar{a})^2.$$

Taking the time derivative we have

$$\dot{V}_g = 2\sum_{1}^{n} \dot{x}_i(a_i - \bar{a})^2 + 4\sum_{1}^{n} x_i(a_i - \bar{a})(\dot{a}_i - \dot{\bar{a}})$$

or

$$\dot{V}_g = 2\sum_{1}^{n} x_i(a_i - \bar{a})^3 + 4\sum_{ij} \dot{x}_i\dot{x}_j a_{ij}.$$

The first term in the right side is the third moment in average effect and will be designated by $\mu_{3(g)}$. The second term reduces to

$$4\sum_{ij} \dot{x}_i\dot{x}_j d_{ij} \text{ or } 4\overline{\alpha_i\alpha_j d_{ij}}$$

so that

$$\frac{dV_g}{dt} = \mu_{3(g)} + 4\overline{\alpha_i\alpha_j d_{ij}}. \tag{6.1}$$

With no dominance the second term vanishes and we have

$$\frac{dV_g}{dt} = \mu_{3(g)}.$$

If the distribution of fitness among individuals in a population is near normal $\mu_{3(g)}$ will be very small as compared with V_g itself. This result seems to indicate that the additive genetic variance is consumed quite slowly by natural selection. This conclusion may not be much influenced in most cases by the presence of dominance, since the second term in (6.1) will usually be a small quantity.

7. APPLICATION TO ARTIFICIAL SELECTION

Here we consider only mass selection with respect to a quantitative character governed by a large number of factors.

Let $P_{ijkl...}$ be the frequency of the ordered genotype $A_iA_jB_kB_l...$, whose phenotypic value or yield is $Y_{ijkl...}$. Applying (3.13), the increase of population mean per generation becomes

$$\Delta\bar{Y} = \sum_{ijkl...} (\Delta Y_{ijkl...}) P_{ijkl...} + 2\left(\sum_i \alpha_i \Delta x_i + \sum_k \beta_k \Delta y_k + ...\right) + \sum \overline{\epsilon \Delta \log \theta}, \tag{7.1}$$

where $\alpha, \beta, ...$, are average effects of the genes on yield at the 1st, 2nd, etc., locus, and ϵ denotes an interaction effect on yield.

The first term in the right side of (7.1) gives the increase of yield by improvement of environment.

In order to handle the remaining terms in the right side of (7.1), we have to establish the relation between the yield ($Y_{ijkl...}$) and the fitness or selective value ($w_{ijkl...}$) of each genotype. For this we will employ the method used by Crow (1955), and based on an earlier finding of Haldane (1932).

Let σ^2 be the total phenotypic variance. Consider various phases in any locus, say the first, and designate the mean genotypic value of individuals with the phase A_iA_j by

$$Y_{ij} = \sum_{kl...} P_{ijkl...} Y_{ijkl...} / \sum_{kl...} P_{ijkl...}.$$

We assume that the means of these phases have very small variance around $\bar{Y}$ compared with σ^2, while individuals with a given phase, say A_iA_j, distribute normally with variance σ'^2 around the mean Y_{ij}.

Let $\tilde{c}$ be the point of truncation measured from the population mean $\bar{Y}$, then the selective value of the phase A_iA_j is

$$w_{ij} = \frac{1}{\sqrt{2\pi}\sigma'} \int_{\tilde{c}}^{\infty} e^{-\frac{(y-\tilde{Y}_{ij})^2}{2\sigma'^2}} dy, \tag{7.2}$$

where $\tilde{Y}_{ij} = Y_{ij} - \bar{Y}$. Now we assume that $(\tilde{Y}_{ij}/\sigma')^2$ is a negligible quantity. Thus we have approximately

$$w_{ij} = S + \frac{\tilde{Y}_{ij}}{\sigma^2} IS \propto 1 + \frac{\tilde{Y}_{ij}}{\sigma^2} I,$$

where

$$S = \frac{1}{\sqrt{2\pi}\sigma} \int_{\tilde{c}}^{\infty} e^{-\frac{y^2}{2\sigma^2}} dy$$

or the proportion saved, and

$$I = \frac{1}{\sqrt{2\pi}\sigma} \int_{\tilde{c}}^{\infty} y e^{-\frac{y^2}{2\sigma^2}} dy / S$$

or the selection differential.

The average excess in fitness of A_i is

$$\tilde{w}_i = \frac{\hat{Y}_{i\cdot}}{\sigma^2} I.$$

Thus the yield is transformed into fitness by multiplying by the factor I/σ^2. Therefore

$$\Delta x_i = x_i \hat{Y}_{i\cdot} \frac{I}{\sigma^2}, \tag{7.3}$$

where $\hat{Y}_{i\cdot}$ is the average excess of the gene A_i on yield. Substituting this in the second term of (7.1) we have

$$2 \sum_i \alpha_i \Delta x_i = 2 \frac{I}{\sigma^2} \sum_i \hat{Y}_{i\cdot} \alpha_i x_i = \frac{I}{\sigma^2} \sigma^2_{g(1)},$$

where $\sigma^2_{g(1)}$ is the additive or genic variance in yield due to the first locus. Thus if we designate by σ^2_g the total genic variance due to all loci, we have

$$2(\sum_i \alpha_i \Delta x_i + \ldots) = \frac{I}{\sigma^2} \sigma^2_g = h^2 I, \tag{7.4}$$

where h^2 is the heritability in the narrow sense.

Next we will consider the effect of gene interactions collectively expressed in the last term of (7.1). The effect of dominance in the first locus appears as

$$\sum_{ij} P_{ij} d_{ij} \Delta \log \theta_{ij},$$

or

$$\sum_{ij} x_i x_j d_{ij} \Delta \theta_{ij}.$$

The most important case of non-random mating is inbreeding in which

$$\theta_{ii} = F/x_i + 1 - F \text{ and } \theta_{ij} = 1 - F \text{ for } i \neq j.$$

For this case, we have

$$\sum_{ij} x_i x_j d_{ij} \Delta \theta_{ij} = \frac{\Delta F}{1 - F} \sum_i d_{ii} x_i - F \sum_i d_{ii} \Delta x_i, \tag{7.5}$$

where Δx_i is given by (7.3). The total contribution by dominance is the sum of such terms for all loci.

Next we will consider terms due to epistasis. As pointed out by Fisher (1918), epistasis involving more than two loci may be rather unimportant and we will consider only dual epistacy. Let $\mathcal{J}_{ab}$ be the epistatic contribution due to interaction between the first and the second loci, then

$$\mathcal{J}_{ab} = \Sigma \overline{\epsilon_{ijkl} \Delta \log \theta_{ijkl}} = \sum_{ijkl} \epsilon_{ijkl} \Delta P_{ijkl},$$

where ϵ_{ijkl} stands for the epistatic effect on yield rather than on fitness. Perhaps the simplest but most important case in which $\mathcal{J}_{ab}$

is not zero is the case in which under random mating the two loci on the same chromosome have not reached linkage equilibrium. Let X_{ik} be the frequency of the chromosome with A_iB_k. Then the frequency of the ordered genotype $A_iA_jB_kB_l$ is

$$P_{ijkl} = \tfrac{1}{2}(X_{ik}X_{jl}+X_{il}X_{jk}).$$

Thus

$$\mathcal{J}_{ab} = 2\sum_{ijkl}\epsilon_{ijkl}\Delta X_{ik}X_{jl} = 2\sum_{ik}\epsilon_{i\cdot k\cdot}\Delta X_{ik}, \tag{7.6}$$

where

$$\epsilon_{i\cdot k\cdot} = \sum_{jl}\epsilon_{ijkl}X_{jl},\ (\epsilon_{ijkl} = \epsilon_{ijlk} = \epsilon_{jikl} = \epsilon_{jilk}).$$

However

$$\Delta X_{ik} = X_{ik}\tilde{w}_{i\cdot k\cdot} - c\sum_{jl}\begin{vmatrix} X_{ik} & X_{il} \\ X_{jk} & X_{jl}\end{vmatrix} w_{ijkl}, \tag{7.7}$$

in which

$$\tilde{w}_{i\cdot k\cdot} = \sum_{jl} w_{ijkl}X_{jl} - \bar{w}$$

and w_{ijkl} is the selective value of $A_iA_jB_kB_l$ while c is the recombination fraction between the two loci. For the derivation of (7.7), the reader may refer to my previous paper (Kimura, 1956). Substituting (7.7) in (7.6) noting that fitness may be obtained from yield by multiplying I/σ^2.

$$\mathcal{J}_{ab} = \frac{I}{\sigma^2}\left\{E - 2c\sum_{ijkl}\epsilon_{i\cdot k\cdot}\begin{vmatrix} X_{ik} & X_{il} \\ X_{jk} & X_{jl}\end{vmatrix} Y_{ijkl}\right\}, \tag{7.8}$$

where

$$E = 2\sum_{ik}\epsilon_{i\cdot k\cdot}\bar{Y}_{i\cdot k\cdot}X_{ik}.$$

The total contribution may be obtained by adding up terms like $\mathcal{J}_{ab}$ for all relevant pairs of loci. The case of random mating with linkage may also be approached by considering additive chromosomal variance rather than additive genic variance.

8. CONCLUDING REMARKS

Any discussion on the change of population fitness must start from the definition of the term fitness. Though there may be diverse ways of defining fitness, the term, to be useful for population genetics, must be related to organism's ability to leave descendants. If it is defined in terms of Malthusian parameters expressing the geometric rate of population increase, the rate of increase of population fitness, as shown from (3.9) through (3.13), is expressed as the sum of three terms : A term due to genic variance, one due to change in environment, and one due to the joint effect of genetic interaction (dominance and epistasis) and change in mating system :

$$\frac{d\bar{a}}{dt} = V_g + \bar{a} + \overline{\Sigma\epsilon\theta}. \tag{8.1}$$

From the standpoint of the genetical theory of evolution, the first term V_g is by far the most important, since it represents the part of increase in fitness due to the improvement of the *genic* constitution of the species. The effects due to the remaining terms are generally speaking unpredictable from the *genic* constitution of the species and may conveniently be treated as environmental factors. However, if we come down to a specific situation, we may handle these terms to obtain more information as shown in section 4.

In this section we will consider the effect of overpopulation on fitness. Since a given niche can support only a finite number of individuals at a given moment, the population mean of the Malthusian parameters, averaged over the long period that the species has lived, must be very near to zero. Whenever the genic constitution of the species is improved by natural selection, the increase in the Malthusian parameters will soon be cancelled out by the increase of population number. Though the equation (8.1) is general enough to include the above process as a special aspect, it may be interesting to separate it for special consideration.

Let M be the Malthusian parameter of an actual population and let N be the population number at a given moment, then we have

$$\frac{1}{N}\frac{dN}{dt} = M \tag{8.2}$$

by definition. Let $a_{ijkl\ldots}$ be the Malthusian parameter of the genotype $A_iA_jB_kB_{l\ldots}$ which would be realised if there were no effect of overcrowding, then $\bar{a}$ is the intrinsic rate of population increase and

$$\frac{1}{N}\frac{dN}{dt} = \bar{a} - f(N), \tag{8.3}$$

where $f(N)$ is a function representing the effect of population number or, when the population is confined to a restricted area, the effect of density. In other words $f(N)$ represents the resistance against population increase due to overcrowding.

From (8.2) and (8.3)

$$M = \bar{a} - f(N)$$

or taking time derivatives of both sides,

$$\frac{dM}{dt} = \frac{d\bar{a}}{dt} - \frac{df(N)}{dt}. \tag{8.4}$$

Since the second term in the right side may be written as

$$\frac{df(N)}{dt} = \frac{df(N)}{dN}\frac{dN}{dt} = MN\frac{df(N)}{dN},$$

(8.4) becomes

$$\frac{dM}{dt} + MN\frac{df(N)}{dN} = \frac{d\bar{a}}{dt}, \tag{8.5}$$

where the right side is given by (8.1). Since we are considering the deleterious effect of *over*population, we may assume that $f(N)$ is a monotone non-decreasing function and therefore $f'(N)$ is non-negative. However, it should be kept in mind that in nature extreme low population density is also disadvantageous for population growth (*cf.* Haldane, 1953).

Returning to (8.3), we will consider a few special cases. For the logistic law of growth

$$f(N) = KN,$$

where K is a constant, and therefore $f'(N) = K$. Thus the second term in the left side of (8.5) becomes KMN.

For weaker resistance, we may take

$$f(N) = K \log N.$$

In this case $f'(N) = K/N$ and we can get rid of a troublesome factor N out of the equation (8.5) :

$$\frac{dM}{dt} + \frac{M}{C} = V_g + \bar{a} + \Sigma\overline{\epsilon\theta}, \tag{8.6}$$

where $C = 1/K$. (8.6) may be considered to be equivalent to Fisher's formulation :

$$\frac{dM}{dt} + \frac{M}{C} = W - D, \tag{8.7}$$

in which " W is the rate of actual increase in fitness determined by natural selection, and D is the rate of loss due to the deterioration of the environment " (Fisher, 1930). W is equal to our V_g and $-D$ corresponds to

$$\bar{a} + \Sigma\overline{\epsilon\theta}.$$

Thus Fisher's " environment " implies not only physical and biological environment in the usual sense, but also genetical environment the effect of which is represented by

$$\Sigma\overline{\epsilon\theta},$$

in our formulation. Considering the great diversity of ways in which non-genic (non-genetic in Fisher's sense) factors come into play, to treat such factors collectively as an environment is quite adequate. However, it is possible, as shown in the present paper, to represent the contribution by such factors in terms of gene interaction and change in mating system in a rather simple way when these are known.

9. SUMMARY

1. When fitness (a) of individual genotypes is measured in Malthusian parameters, the rate of increase of population fitness due to natural selection is expressed as a sum of the three terms : (i) a term due to *genic* (or additive genetic) variance in fitness (V_g), (ii) one due

to change in environment, and (iii) one due to joint effect of genetic interaction (dominance and epistasis) and change in mating system. Thus we have

$$\frac{d\bar{a}}{dt} = V_g + \overline{\frac{da}{dt}} + \Sigma\epsilon\,\overline{\frac{d}{dt}\log\theta},$$

in which ϵ stands for an interaction effect in fitness and θ is the corresponding coefficient of departure from random combination. The summation extends over all possible interactions. The above formulation establishes the roles of additive, dominance and epistatic components on the rate of increase of population fitness. It is the reformulation of the results published by Fisher in 1930 under the title *The Fundamental Theorem of Natural Selection*, and in no way differs from it in substance. θ corresponds with λ of Fisher's (1941) treatment.

2. An attempt to formulate the law of change in gene frequencies due to natural selection has led to a theorem which, for a simple case of random mating with constant fitness of individual genotypes, may be expressed in the following form : For a given short time interval δt, the natural selection causes gene frequency changes, $\delta x_1, \delta x_2, \ldots, \delta x_n$, in such a way that the increase of population fitness, $\delta\bar{a}$, shall be maximum under the restriction of

$$\sum_{i=1}^{n} \frac{(\delta x_i)^2}{x_i} = \tfrac{1}{2} V_g (\delta t)^2.$$

This theorem is extended to cover a quite general situation. It is remarkable that the set of differential equations describing the law of change in gene frequencies by natural selection can be derived from the theorem. It may be called " a maximum principle in the genetical theory of natural selection " analogous to the principle of least action in physics.

3. The rate of change of the genic variability in fitness has been studied for a simple situation. The result seems to indicate that the genic variance is consumed rather slowly by natural selection.

4 The rate of increase of yield by artificial selection has been given with special reference to the roles of dominance and epistatic components when inbreeding and linkage are involved.

5. The present formulation on the change of population fitness is compared with the results by Fisher (1930) and the effect of population number on fitness has been discussed.

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GENE FREQUENCIES IN WILD POPULATIONS OF *TRIFOLIUM REPENS* L.

III. WORLD DISTRIBUTION

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1. INTRODUCTION

ALTHOUGH there have been a great number of publications describing morphological and cytological adaptation, Dobzhansky (1951) has stated that " the extent of the diversity of physiological and biochemical traits in living beings is still quite imperfectly known ". The aim of this investigation was to establish the pattern of gene controlled chemical variation in world populations of *Trifolium repens*.

It is already known that *T. repens* possesses cyanogenetic glucosides consisting of 80 per cent. lotaustralin and 20 per cent. linamarin (Melville and Doak, 1940) and their hydrolysing enzyme linamarase (Coop, 1940). One of the products of hydrolysis is HCN. This was believed to cause bloat or poisoning effects in ruminants but Corkill (1952) was unable to demonstrate deleterious effects when sheep were grazed on high cyanogenetic glucoside lines of *T. repens*.

The presence of these cyanogenetic glucosides and their hydrolysing enzyme in *T. repens* is determined by independent single genes (Corkill, 1942 ; Atwood and Sullivan, 1943) while modifying genes determine the quantity of cyanogenetic glucosides produced (Corkill, 1942). In the following pages the term " lotaustralin " is used instead of cyanogenetic glucosides and " linamarase " refers to the hydrolysing enzyme. Previous investigations (Daday, 1954*a*) demonstrated that the frequencies of the lotaustralin and linamarase genes varied from 0 to 100 per cent. in European and Near Eastern wild populations of *T. repens*. A similar gene frequency cline was observed in Alpine populations (Daday, 1954*b*). Winter temperature was considered to be the major factor responsible for the formation of these gene frequency clines in wild populations.

The major part of this investigation was carried out at the Welsh Plant Breeding Station, Aberystwyth, Great Britain and completed at C.S.I.R.O., Canberra.

2. METHODS

The presence of lotaustralin and linamarase in plants was shown by the Guinard picric acid paper test (Corkill, 1940) with certain modifications (Daday, 1954*a*). According to their chemical constituents the plants were classified into four chemical phenotypes : (1) Lotaustralin and linamarase (*AcLi*) ; (2) Lotaustralin only (*Acli*) ; (3) Linamarase only (*acLi*) ; (4) Neither lotaustralin nor linamarase (*acli*). (Appendix I).

The gene frequencies were calculated by the Hardy-Weinberg formula.

The collection of seed samples was organised by the British Council, the United States Department of Agriculture, and Universities and Agricultural Experiment Stations of various countries. The technique of collection has been described in a previous paper (Daday, 1954*a*).

3. GEOGRAPHICAL DISTRIBUTION OF *T. REPENS*

T. repens is a typical species of the temperate regions of the world. However, the high adaptability of the species enabled it to extend to the Arctic regions (Murmansk, Kanin Peninsula, U.S.S.R.) and to the sub-tropical regions as in Queensland (Australia). The geographical distribution of *T. repens* is presented in fig. 1.

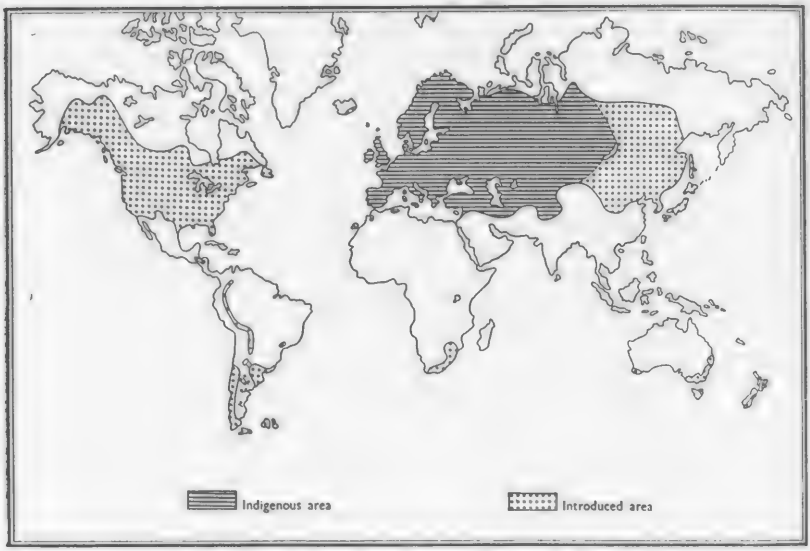

FIG. 1.—Geographical distribution of *Trifolium repens* L.

The Mediterranean region is considered by Vavilov (1951) to be the centre of origin of *T. repens*. This species has spread all over Europe and has also invaded the larger part of Asia by means of animal, human and spontaneous distribution. Its occurrence is recorded on the Asiatic mainland (Komarovii, 1945) as follows: Turkey, Lebanon, Israel, Iraq, Persia, Pakistan, India (Himalaya Range up to 20,000 feet), U.S.S.R., China, Mongolia and Korea. It is considered that the indigenous area consists of the whole European and the western half of the Asiatic distribution area, whilst east from the Baikal Lake is the recently occupied region. Personal information obtained from Japan indicates that the first sample of *T. repens* was carried by a Dutch vessel which arrived at Nagasaki in 1846. This is considered as one of the ancestral sources of the Japanese *T. repens*.

This species is indigenous to North Africa. It occurs both in low

land and at high elevations (Atlas Mountains) in Morocco and Tunisia wherever sufficient soil moisture is available. One collection came from an altitude of 7500 feet. The plant was introduced into South Africa, establishing itself in Cape Province and in cool mountain areas where the rainfall is evenly distributed and along stream banks in Natal, Transvaal, and Pretoria. Botanical surveys indicate the presence of the species in natural vegetation on the Canary Islands.

T. repens was introduced into North America by the earliest settlers and now it is extremely abundant in most of the settled areas. It is one of the most widespread species and extends northward to Canada and Alaska. It has invaded the whole U.S.A. but between the Cascade, Sierra Nevada mountains and the 100° parallel, grows along rivers, around habitations and irrigated fields. According to a personal communication, *T. repens* was carried to Alaska in about 1920.

The distribution of introduced *T. repens* is restricted to the temperate regions in Central and South America. It occurs in the high altitudes of the Andes and other mountainous regions in Guatemala, Costa Rica, Venezuela (up to 9000 feet), Columbia, Peru, and Bolivia. Comparatively recent introduction started the spread of this species in Brazil (Rio Grande do Sul, Minas Gerais). It is more common in Chile, and in the humid parts of Argentina and some fertile regions of Uruguay.

T. repens is not indigenous in Australia and New Zealand. The spontaneous spread of this species is mainly restricted to humid coastal regions of Australia, but is often seen along streams, and under irrigation in the drier areas. This species was introduced as a pasture plant during the early history of New Zealand and was used to oversow cleared forest areas, bush burns, and *tussock* grassland areas. Now it has become the most important legume component of the grassland vegetation but it also appears naturally along roadsides and where the soil has been disturbed.

4. GENE FREQUENCIES IN WILD POPULATIONS OF THE WORLD

A survey of lotaustralin and linamarase gene frequencies (Daday, 1954*a*) in European and Near Eastern *T. repens* populations disclosed a cline of dominant gene frequency from the warm winter region, where high frequencies predominate, to low frequencies which are most common in Northern Europe. This species is considered to have spread by natural means to the largest part of the Asiatic distribution area.

Four samples were tested (Appendix I) from the mainland of Asia. The southern populations exhibited a higher frequency (Iran, Karaj river area, *Ac* 100·0, *Li* 40·6 per cent. ; India, Almora, *Ac* 67·1, *Li* 73·2) than those collections derived from U.S.S.R. (Turgatuj and Novosibirsk, *Ac* 0·0, *Li* 0·0).

Seven samples represent the Japanese *T. repens* populations. Four of these originated from the southern warm winter regions and three from the cold winter regions. The warm winter region collections are from the South Pacific Coast Region (Miyazaki and Kochi), Seto-Inland—Sea Coast Region (Zentsuji) and the Kanito region with a dry winter climate (Konosu collection). They represent rather mixed gene frequencies (*Ac* 14·7-52·3, *Li* 18·8-42·3 per cent.). The remaining three samples are from the Sea Coast Region in a cool winter area of Japan, Shindo-Mura (*Ac* 54·7, *Li* 21·5 per cent.) ; the cold area of the Pacific Coast Tohoku (North Eastern) Region represented by the Shimo-Kuriyagawa collection (*Ac* 26·6 ; *Li* 28·0 per cent.) ; and the coldest winter region of Japan, Hokkaido in the north, where *Ac* 15·0, *Li* 23·2 exhibit the lowest frequencies in the Tsukissapau sample. The Japanese samples may thus be divided into those from the southern warm winter region with mixed gene frequencies, and those from the northern colder winter area with decreasing gene frequencies of *Ac*.

T. repens is indigenous to North Africa, where it shows some indication of altitudinal gene frequency clines (Rabat, near sea level, *Ac* 100·0, *Li* 100·0 ; Tirsal, at 4500 feet, *Ac* 100·0, *Li* 100·0 ; Oukaiemden, at 7500 feet, *Ac* 59·7 and *Li* 69·5 per cent.). All the samples tested from South Africa varied between 51·7 and 91·0 per cent. for *Ac*, and 43·0 and 70·2 per cent. for *Li*. There is no evidence for gene frequency cline formation in South Africa.

The U.S.A. and Canadian populations are represented by considerable numbers of *T. repens* samples from Louisiana to Alaska. A high mean lotaustralin gene frequency was found in most of the samples above the 40° F. January isotherm (Baton Rouge 71·9, New Orleans 75·8, San Francisco 61·4, San Francisco 84·3, Rio Monte 100·0 for *Ac* gene frequencies). Between the 40° and 0° F. isotherm, there are collections from Oregon, Ohio, Nebraska, Illinois, Minnesota, Alaska, Quebec and Alberta which exhibit rather mixed gene frequencies with respect to the *Ac* gene. However, 32·6 to 2·2 per cent. frequencies were found below the 0° F. line (Brandon, Fairbanks). This relationship between the January mean temperature of localities and gene frequencies (fig. 2) is highly significant ($P<0{\cdot}001$).

The regression slope indicates that the change in the *Ac* frequency is 0·681 per cent. per 1° F. mean temperature. The relationship between *Li* gene frequencies and temperature change is not significant. Natural selection seems to have occurred in North America and a frequency cline for the *Ac* gene has already formed. The occurrence of a *Li* frequency cline in North America does not seem to have any statistical support.

South American collections were obtained from Peru, Brazil and Argentina. Gene frequencies vary considerably, 7·9-100·0 per cent. for the *Ac* gene and 13·0-100·0 per cent. for the *Li* gene. The lowest *Ac* frequency (7·9 per cent.) was found at Pedro Leopoldo in Minas

Gerais, Brazil, where the winter temperature is characterised by being the highest (60° F.) among the sampled areas. High *Ac* frequencies were established at Esquel, Argentina, where the mean temperature is the lowest among the samples (30° F.). The mean July temperatures of these South American localities are comparatively high (30-60° F.) and no collection was obtained from regions having July mean temperatures below 30° F. The absence of a gene frequency cline might reflect the absence of a large range in winter temperature in the parts of South America studied.

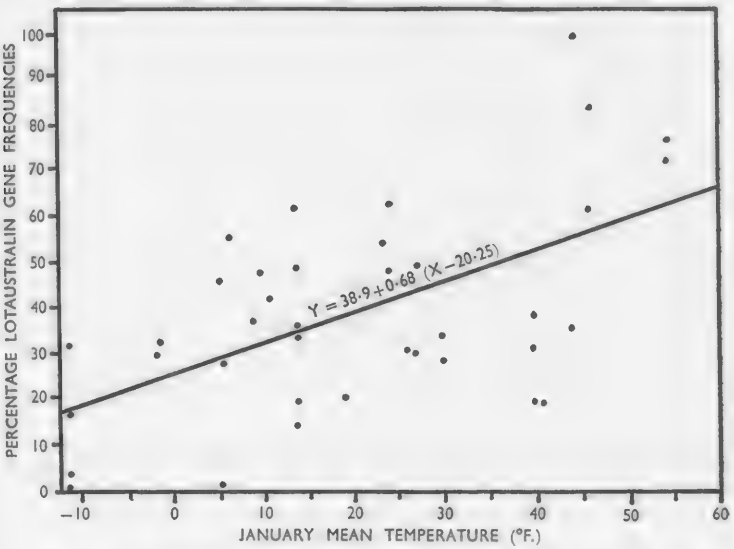

FIG. 2.—Correlation between lotaustralin gene (*Ac*) frequencies and January mean temperature in North American wild populations of *T. repens*.

Australian populations were represented by collections from old established pastures of New South Wales, Victoria, Tasmania, South Australia, and the Australian Capital Territory. Apart from the low land collections, altitudinal samples (Mt. Kosciusko, Mt. Gingera) were also tested. The frequencies of *Ac* range from 0·0 to 75·1 per cent. and those of *Li* from 6·1 to 78·8 per cent. in low land populations. There was no pattern or indication of cline formation. Mt. Kosciusko is in New South Wales and has the coldest winter in Australia. The highest elevations are covered by snow for 6 to 8 months of the year. Collections were made at altitudes from 3000 to 5800 feet. *Ac* frequencies were uniformly low (3·2 to 8·2 per cent.) but variable proportions of *Li* frequencies (28·6 to 51·2 per cent.) were found in this region.

The North and South Islands of New Zealand were represented by four collections. Hastings exhibited 100·0 per cent. frequency for the *Ac* gene (*Li* 88·0), while the lowest frequency, 37·7 per cent. for *Ac* (*Li* 70·0 per cent.), was at Winston. Other *Ac* values varied between these extremes.

The samples collected in Japan, South Africa, South America, Australia and New Zealand appear to be rather heterogeneous. The χ^2 test for heterogeneity has been carried out for samples of each of the above geographical areas. The heterogeneity test proved to be highly significant for all the samples with the exception of *Li* containing plants in New Zealand.

The geographical distribution of the lotaustralin gene frequencies in *T. repens* populations of the world is illustrated in fig. 3. Both Europe and the mainland of Asia show a 100-0 per cent. decrease. This gradual diminishing in gene frequencies occurs across the January isotherms. Above the 50° F. isotherm all populations have 100·0 per cent. dominant *Ac* frequency. Below the 50° F. isotherm the gene frequencies fall from 100·0 to 5·0 per cent. at the 30° F. isotherm. The rest of the area has 5 per cent. or lower frequencies. In North America, 100·0 to 60·0 per cent. was the most common frequency above the 40° F. isotherm, whilst below the 0° F. isotherm, dominant gene frequencies below 30·0 per cent. prevailed. *Ac* gene frequencies in northern Japanese populations also show a tendency to diminish with decreasing winter temperature. With the exception of mountainous regions with cool winters, mixed gene frequencies have been found all over the southern hemisphere.

Linamarase gene frequencies are presented in fig. 4. As with *Ac*, the *Li* frequency variation occurred within a range of 100 per cent. in Europe and Asia. More variable than *Ac*, but still high, were the frequencies of *Li* above 50° F. The decrease at lower temperatures was not as sharp as with the *Ac* gene. The mean frequency of *Li* is 15 per cent. at the 30° F. isotherm and then it gradually falls to 0 per cent. No cline has yet been found in recently colonised areas of the northern hemisphere. Mixed populations have also been found in the southern hemisphere.

The quantity of lotaustralin and linamarase produced by plants varies considerably. Corkill (1942) showed that modifying genes determined the amount of lotaustralin synthesised by plants. The relationship between the *Ac* gene frequencies and the mean rating of lotaustralin produced by world *T. repens* populations is presented in fig. 5.

Association between *Ac* frequencies and mean rating was examined by classifying the data into a 3×3 contingency table with the boundaries defined by dividing the range of each variable into thirds. χ^2 is 40·41 with 4 df ($P < 0{\cdot}001$) so that an association clearly exists.

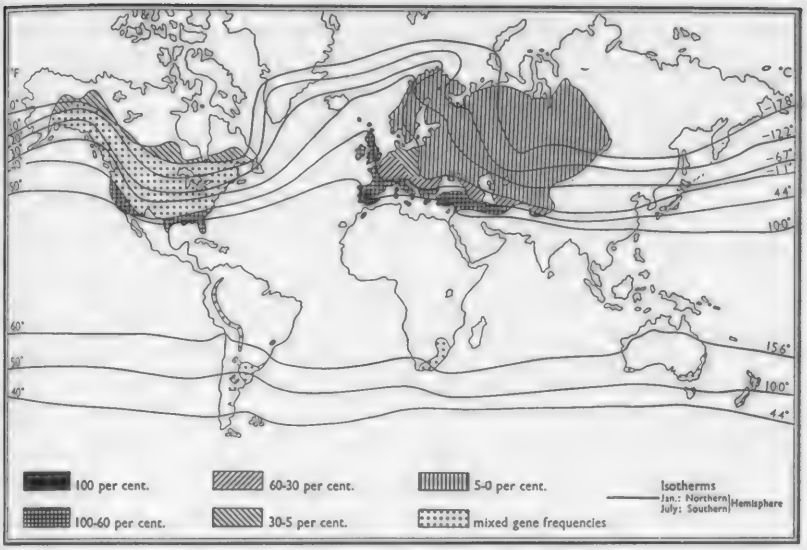

FIG. 3.—Distribution of lotaustralin gene frequencies in world populations of *Trifolium repens* L.

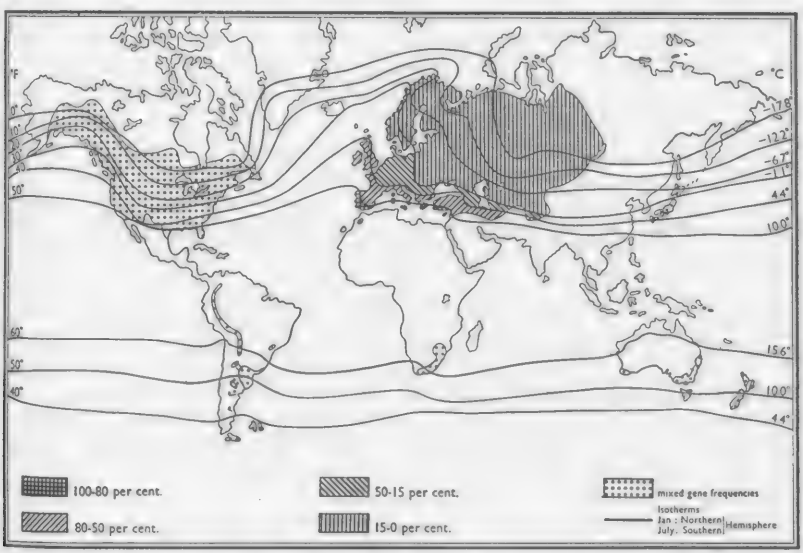

FIG. 4.—Distribution of linamarase gene frequencies in world populations of *Trifolium repens* L.

5. DISCUSSION

Huxley (1955) has emphasised the evolutionary significance of intraspecific differentiation. He stated that genic differences between populations may enable the species to occupy areas which environmentally are sharply distinct. If there is adaptation to a continuous range of environments, the formation of a ratio-cline will be apparent where a balanced equilibrium exists between the adaptive morphism

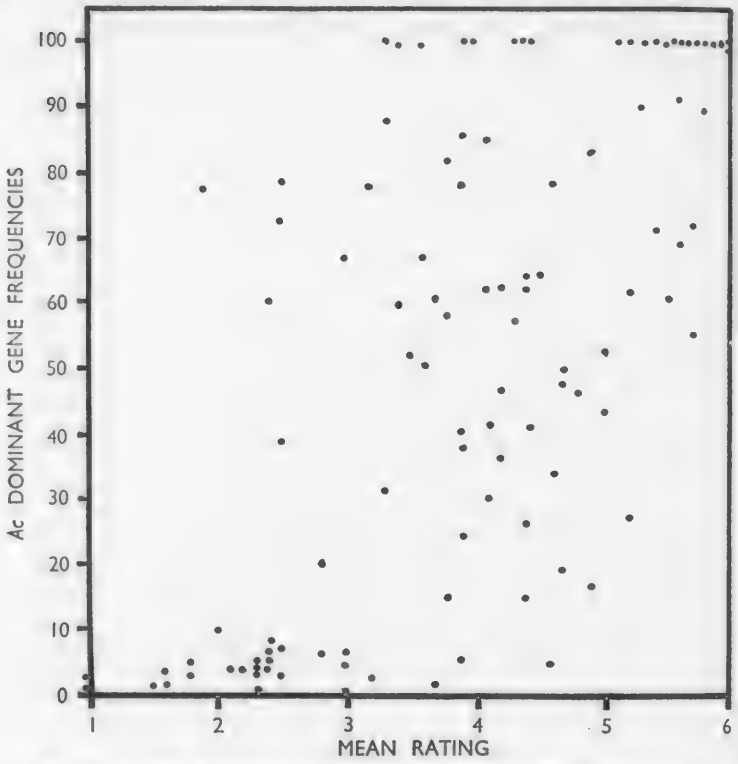

FIG. 5.—Correlation between lotaustralin (*Ac*) gene frequencies and mean rating of produced lotaustralin in world populations of *T. repens*.

and the environment. The investigation of ratio-clines may lead to the exploration of selective forces. A typical example of a ratio-cline has been demonstrated in *T. repens*. The dominant lotaustralin and linamarase gene frequencies decrease from 100 to 0 per cent. as the mean winter temperature decreases. Therefore, winter temperature is considered as the major selective force in this example. It can also be seen that the gene frequency cline is more evident in those regions where the coldest month of the year has a temperature range below 40° F. This range of required winter temperature can

be found in the northern hemisphere but not in the southern hemisphere with the exception of such cold winter mountainous regions as Mt. Kosciusko.

The occurrence and spread of a mutant gene can influence considerably the selective advantage or disadvantage of a population, as is well illustrated in *Pisum arvense* L. (Scheibe, 1955). A pair of genes governs the presence or absence of a waxy layer on the leaves of *P. arvense*. A thick layer of wax is found on plants of dry climatic regions such as Asia Minor and the European Continent while populations have been found in the British Isles with thin waxy layers. Scheibe studied the behaviour of genotypes differing only in the waxy character under competition in a low rainfall area in Germany where a mixture consisting of 50 per cent. of each genotype was grown for four years. The waxy genotype increased to a frequency of 80·9 per cent. while the non-waxy genotype was reduced to 19·1 per cent. of the original population. The non-waxy phenotype was associated with a higher rate of transpiration which in turn reduced seed number and seed weight. This example shows that single gene mutants can contribute considerably to the selective potential of a species.

The distribution of allelic frequencies in populations according to selection pressure is well demonstrated in *Ricinus communis* L. and species of *Eucalyptus*. A single dominant allele *B* produces waxy bloom on the stem of *R. communis* (Harland, 1947). The survey of *B* gene frequencies in populations of Peru shows a gradual increase in the dominant allele with altitude. Plants carrying the *B* gene failed to fruit except when growing in dry sunny conditions, but the *bb* genotype showed normal fruiting under coastal, foggy conditions during the winter. This ratio-cline clearly indicates that the *B* gene or some closely linked genes have some physiological disadvantage under humid conditions. Similar clinal variation was disclosed in *Eucalyptus gigantea*, *E. gunnii* and *E. coccifera* by Barber (1955). The appearance of glaucous stems and leaves shows a considerable clinal variation in *Eucalyptus* along a mountain transect from Golden Valley at 1200 feet to Great Lake at 3500 feet in Central North Tasmania. Within a species, the non-glaucous type is most common at low elevations, while increased glaucousness frequencies are found at higher elevation. According to Barber, the abundance of glaucous trees is correlated with changes in frost activity.

If one of the alleles becomes disadvantageous in a population, natural selection will rapidly reduce its frequency. This rapidity of natural selection is well illustrated in moths (Lepidoptera) (Ford, 1955). The dominant melanic allele of the moth has rarely been found in moth species of non-industrial areas. However, a number of species have become fully, and other species considerably, darker melanics during the last 100 years in the industrial areas of Great Britain and Western Europe. The influence of natural selection is

also indicated in *T. repens* populations of North America and Japan. The introduced *T. repens* is slowly adapting to these new environments by forming gene frequency clines. Comparison can be made between the gene frequency clines of indigenous (European, Near Eastern) and introduced (North American) *T. repens* populations through their regression lines (fig. 6). The increase of gene frequencies per 1° F. of January mean temperature is 4·23 per cent. for *Ac* and 3·15 per cent. for *Li* in indigenous populations, while the value for *Ac* falls to 0·68 per cent. in North American populations. In the European and Asiatic (mainland) regions, which are the oldest distribution areas, it can be considered that there is a balanced equilibrium

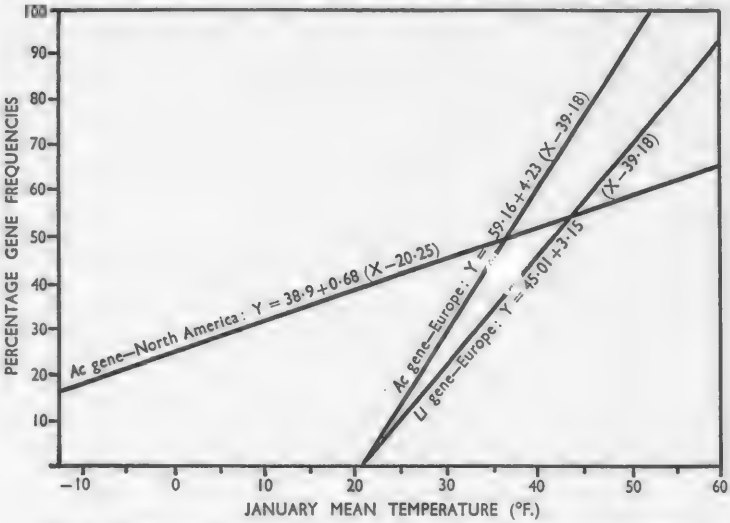

FIG. 6.—Comparison between the regression lines of gene frequencies in European and North American wild populations of *T. repens* L.

between gene frequencies and January mean temperature. On the other hand, gene frequencies and January mean temperature have not yet reached an equilibrium in the North American population. It is postulated that the American gene cline is gradually moving under selection pressure and its regression line will occupy the same position as the European regression line when equilibrium is reached during the course of evolution. The postulate that natural selection is acting to form gene frequency clines in *T. repens* populations in North America has also been supported by the evidence of Portz and Jackobs (1955). Four thousand four hundred and fourteen plants of *T. repens* cultivated Ladino, have been tested from a geographical range between Washington and California. The frequencies of plants containing lotaustralin varied from 67 to 0 per cent. between seed lots of certified Ladino strains. A typical cline from south to north

(high-low lotaustralin plant frequencies) has been disclosed. The original Ladino strain was found to contain $5{\cdot}2 \pm 1{\cdot}95$ per cent. lotaustralin genes (Daday, 1954*c*) and this increase is partly due to natural selection in U.S.A.

In spite of the fact that several single gene controlled enzymes are known in micro-organisms, no single gene controlled enzyme has been reported in flowering plants other than linamarase in *T. repens*. Six different quantities of enzyme production have been indicated (Daday, 1954*b*). It is still unknown whether the different quantities of linamarase are controlled by a series of alleles or by modifying genes.

The presence together of the products of the genes *Ac* and *Li* determine the *in vitro* production of HCN ; but, as suggested to the author by his colleague Dr F. H. W. Morley, it cannot be assumed that it is the product of this chemical reaction which is recognised by the forces of natural selection. The presence of several populations in which *Ac* is at low frequency and *Li* at high frequency and vice versa (*e.g.* in North America, Fairbanks : *Ac* 4·2, *Li* 38·6 per cent. ; Brandon : *Ac* 29·3, *Li* 81·1 per cent. ; and also the fact that in Europe *Ac* and *Li* regression coefficients are significantly different, $t(86) = 2{\cdot}206$, $P = 0{\cdot}05\text{-}0{\cdot}02$) suggests that selection acts on these genes or their products independently. The presence of frequency clines in the European populations (Daday, 1954*a*) indicates that both genes are subject to agents of selection associated with temperature, but in the absence of further information, nothing more may be deduced.

These findings underline an important principle. Although the particular action of a gene product may be known, it is not necessarily this action which is the subject of selection under natural conditions.

The old naturalists (*e.g.* Kerner *et al.*, 1894) emphasised the protective function of alkaloids and other substances in flowering plants. The presence of lotaustralin in *T. repens* may afford protection against the feeding of such animal predators as rabbits and snails, but probably the major role is exerted through a physiological process as yet not understood.

The present investigation has shown that *T. repens* has formed a balanced gene frequency cline in its indigenous distribution areas, and that natural selection is still acting to establish equilibrated gene frequency clines in the areas of introduced populations wherever the selective force, low winter temperature range, exists.

6. SUMMARY

1. The distributions of lotaustralin (*Ac*) and linamarase (*Li*) gene frequencies in wild populations of *T. repens* are presented.

2. Gene distribution :

(*a*) In Europe, North Africa and the mainland of Asia, there are 100 to 0 per cent. gene frequency clines.

(*b*) In North America and Japan, decreases in *Ac* gene frequencies exist from South to North.

(*c*) In South America, South Africa, Australia and New Zealand, gene frequencies exhibit an irregular pattern.

3. The decreasing gene frequencies were found to be highly correlated with decrease in mean winter temperature.

4. There is a relationship between gene frequency and quantity of gene products.

5. It is apparent that natural selection has already produced a balanced equilibrium in Europe and the mainland of Asia, and that natural selection is still in progress in North America and Japan.

6. Formation of *Ac*, *Li* gene frequency clines are not expected in the southern hemisphere with the exception of mountainous regions.

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[Appendix

APPENDIX I

Phenotypes and gene frequencies in wild populations of Trifolium repens

Localities	Phenotypes				Genes		January * mean temperature (° F.)
	No. of plants				Percentages		
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
ASIA							
India							
Almora	94	5	9	3	67·1	73·2	54·0
Iran							
Karaj River area	101	56	0	0	100·0	40·6	40·0
U.S.S.R.							
Turgatuj Baikal Region	0	...	...	68	0·0	0·0	−4·6
Novosibirsk	0	...	...	58	0·0	0·0	−2·9
Japan							
Miyazaki	16	24	34	73	14·7	18·8	44·2
Kochi	56	46	14	16	52·3	31·5	43·0
Zentsuji	23	29	42	73	17·0	21·8	42·8
Konosu	75	26	25	24	42·8	42·3	39·6
Shindo-Mura	49	75	11	21	54·7	21·5	34·5
Shimo-Kuriyagawa	27	38	41	35	26·6	28·0	28·2
Tsukissapau	22	18	37	67	15·0	23·2	20·7
AFRICA							
Morocco							
Rabat	124	0	0	0	100·0	100·0	54·5
Tirsal (4500 feet)	42	0	0	0	100·0	100·0	...
Oukaiemden (7500 feet)	67	5	11	3	59·7	69·5	...
							July mean temperature (° F.)
Union of South Africa							
George, Cape	79	13	2	0	85·4	62·8	55·0
Humensdrop, Cape	49	9	8	3	60·1	58·3	55·0
Escourt, Natal	43	3	10	4	51·7	65·8	55·0
Bloemfontein, Orange Free State	60	10	8	5	60·4	57·6	50·0
Pretoria, Transvaal	83	39	0	1	91·0	43·0	50·0
Potchefstroom, Transvaal	62	4	10	3	59·4	70·2	50·0
							January mean temperature (° F.)
NORTH AMERICA							
U.S.A.							
Baton Rouge, La.	104	1	8	1	71·9	86·8	54·6
New Orleans, La.	112	0	7	0	75·8	100·0	54·6

* Data obtained from individual meteorological stations and also from C.S.I.R. (1933), Clayton (1927).

APPENDIX I—*continued*

Localities	Phenotypes: No. of plants				Genes: Percentages		January mean temperature (° F.)
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
San Francisco, Cal.	87	10	12	5	61·4	63·7	47·2
San Francisco, Cal.	72	7	2	0	84·3	70·6	47·2
Guerneville, Cal.	62	1	40	6	35·0	74·7	43·7
Rio Monte, Cal.	119	0	0	0	100·0	100·0	44·0
Woodburn, Ore.	14	5	18	18	19·1	35·3	40·0
Albany, Ore.	3	18	5	34	19·4	6·9	40·0
Shedd, Ore.	18	4	11	9	31·0	44·4	39·2
Hubbard, Ore.	33	8	19	6	38·4	54·0	39·2
N.E. Oregon, Ore.	38	27	19	32	33·7	28·7	30·0
N.E. Oregon, Ore.	18	1	16	5	27·5	61·3	30·0
Union, Ore.	39	1	30	8	30·2	66·0	26·0
Lat. 41° 14′ 30″ Long. 81° 18′ 0″ Ohio	43	41	9	21	46·7	26·2	26·9
Lat. 41° 14′ 30″ Long. 81° 28′ 0″ Ohio	18	36	10	42	30·0	14·2	26·9
Lat. 40° 55′ Nebr. Long. 96° 10′ Nebr.	35	62	5	10	63·4	19·8	23·7
Chicago, Ill.	71	24	11	14	54·4	43·7	23·6
St Paul, Minn.	5	16	10	50	13·9	9·7	13·1
St Paul, Minn.	15	19	19	47	18·8	18·8	13·1
Mandam, N. Dak.	38	33	28	18	37·3	34·0	9·0
Homer, Alaska	31	13	11	6	47·2	44·2	23·7
Homer, Alaska	11	10	15	21	20·5	26·2	19·0
Matanuska Valley, Alaska	23	15	18	10	34·9	38·4	13·0
Palmer, Alaska	22	7	13	11	32·7	41·7	13·0
Fairbanks, Alaska	21	21	21	15	32·1	32·1	−11·0
Fairbanks, Alaska	3	2	35	21	4·2	38·6	−11·0
Fairbanks. Alaska	11	1	22	4	17·3	63·7	−11·0
Fairbanks, Alaska	3	1	42	46	2·2	28·5	−11·0
Canada							
Lennoxville, Que.	90	2	31	3	48·1	80·1	14·0
Lennoxville, Que.	96	2	17	0	61·6	86·8	14·0
St Anne de la Pocatire, Que.	71	10	26	7	46·2	61·4	10·0
Grandes Bergeronnes, Alb.	71	8	32	9	41·5	62·4	11·8
Lacombe, Alb.	69	9	18	1	55·7	67·9	7·4
Lacombe, Alb.	21	2	20	5	27·8	61·8	7·4
Peace River area, Alb.	53	29	15	19	45·9	35·7	6·0
Peace River area, Alb.	0	1	30	87	0·4	13·6	6·0
Brandon, Man.	27	9	21	9	32·6	47·8	−1·6
Brandon, Man.	13	1	14	0	29·3	81·1	−1·6
							July mean temperature (° F.)
SOUTH AMERICA							
Peru							
Cuzco	165	0	0	0	100·0	100·0	30·0
Brazil							
Curitiba	27	84	0	0	100·0	13·0	54·3
Pedro Leopoldo, area Minas Gerais	11	4	48	36	7·9	36·4	60·0

APPENDIX 1—*continued*

Localities	Phenotypes: No. of plants				Genes: Percentages		July mean temperature (° F.)
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
Argentina							
Monte, Prov. Buenos Aires	76	7	17	4	55·1	67·5	48·9
Las Rosas, Prov. Santa Fe	122	12	13	7	64·0	64·9	50·8
Esquel, Gov. of Chubut	46	45	7	7	63·5	29·6	30·0
Sarmiento, Gov. Comorodo Rivadavia	21	28	30	61	19·4	20·3	37·8
AUSTRALIA							
Bateman's Bay, N.S.W.	6	2	72	23	4·0	50·7	53·0
Bodalla, N.S.W.	78	3	14	3	58·4	75·3	51·9
Central Tilba, N.S.W.	1	0	78	22	0·5	53·3	51·9
Central Tilba, N.S.W.	1	0	84	4	0·6	78·8	51·9
Borogo, N.S.W.	59	4	24	16	37·7	55·9	48·7
Bega, N.S.W.	37	7	21	2	41·4	63·4	48·7
Bega, N.S.W.	0	0	29	44	0·0	22·4	48·7
Bega, N.S.W.	10	0	76	21	4·8	55·7	48·7
Tathra, N.S.W.	12	0	44	51	5·8	31·0	48·7
Nimmitabel, N.S.W.	0	1	37	35	0·7	29·8	36·7
Butlers, N.S.W.	7	0	51	44	3·5	43·3	...
Big-Bog, N.S.W.	8	2	60	35	4·9	40·6	...
Melbourne, Vic.	61	8	15	61	27·6	31·0	50·3
Somers, Vic.	19	9	11	7	37·4	41·0	48·8
Pomborneit, Vic.	8	12	6	92	8·9	6·1	50·0
Mt. Gambier, S.A.	44	9	36	33	24·8	41·3	49·1
Mt. Gambier, S.A.	16	22	20	56	18·3	17·3	49·1
Hd. Benara, S.A.	99	7	5	2	75·1	71·8	...
Hobart, Tas.	84	6	5	3	71·4	69·7	46·6
Ranelagh, Tas.	85	1	30	7	45·1	74·5	43·0
Mt. Kosciusko Region							
Maffra, 3000 feet	4	2	43	47	3·2	28·6	...
Jindabyne, 3000 feet	7	6	84	39	4·9	42·5	...
Hotel Kosciusko, 5200 feet	8	1	78	30	3·9	48·5	31·9
Munyang, 4600 feet	6	2	92	42	2·9	44·3	...
The Smiggin Holes, 5500 feet	12	2	84	28	5·7	51·2	...
Spencer's Creek, 5700 feet	2	4	20	12	8·2	35·1	...
The Chalet, 5800 feet	11	5	84	53	5·4	38·4	26·5
Mt. Gingera							
Mt. Gingera, A.C.T., 5973 feet	0	0	83	2	0·0	84·7	...
Mt. Gingera, A.C.T., 5973 feet	2	0	16	39	1·8	17·3	...
NEW ZEALAND							
Winston	70	4	41	7	37·3	70·0	48·8
Hastings	68	1	0	0	100·0	88·0	55·1
Nelson	44	3	3	3	66·4	66·4	54·2
Palmerston North	30	1	7	4	48·8	65·5	53·1

STUDIES ON *PERIPLANETA AMERICANA*

III. SELECTION FOR HETEROZYGOSITY

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1. INTRODUCTION

In an earlier account we described the occurrence of interchange heterozygosity in a wild isolated population of the American cockroach from a coal mine in Treharris, Glamorgan and in a laboratory culture established from this population. As a result of these investigations it was suggested that the wild population included two mating groups (Lewis and John, 1957).

The present account arises from an extended investigation of the Treharris population which supports this suggestion. It also deals with an analysis of material from seven other sources, two wild populations and five laboratory cultures. The details of these populations are given in table 1 and we are indebted to the persons mentioned there for supplying material.

Interchanges do not arise very often and the individuals in which they originate are always heterozygous for the change. In the absence of selection, inbreeding is expected to reduce the frequency of heterozygotes. Therefore, the high frequency of interchange heterozygotes in these cockroach populations, all of which are inbred, must indicate that the structural hybrids are at a selective advantage. Selection for genic heterozygosity is revealed by interchange hybridity (Darlington and La Cour, 1950).

The chromosome number of *P. americana* is 33 ♂ and 34 ♀. All the analyses were made on adult male imagines and as the X chromosome is not involved in any of the interchanges it has been omitted in the tabulation of data. The cytological methods have been described elsewhere (John and Lewis, 1957).

2. THE INCIDENCE OF INTERCHANGE HETEROZYGOTES

In addition to structural homozygotes, individuals heterozygous for one, two and three interchanges have been found. (plate, figs. 4-9). The distribution of the various karyotypes in the ten localities studied is given in table 2. A χ^2 test showed no significant difference between the wild population and the laboratory culture totals when all classes were considered (table 3). But when the ratio of homozygotes to heterozygotes was tested the frequency of homozygotes was found to

TABLE 1

Sources of material

Population	Prefix	Origin and remarks
(*a*) Wild		
Tonypandy . .	P	Anthony and Pandy Colliery, Glamorgan; sunk in 1875
Cilfynydd . .	C	Cilfynydd Colliery, Glamorgan; sunk in 1888
Treharris, location 1	T 1	Two distinct breeding units from the Deep Navigation Colliery, Treharris, Glamorgan; sunk in 1873
Treharris, location 2	T 2	
(*b*) Culture		
Treharris culture .	TC	Culture established from Treharris mine (T 1 and T 2 mixed) in 1946
Manchester . .	M	Culture established from individuals collected at Manchester Zoo, subsequently mixed with material from Biological Supply Agencies. Donor, Prof. R. G. Dennell, Dept. Zoology, Manchester Univ.
Dundee . .	D	Sub-cultured from Manchester population, no subsequent mixing. Donor, Prof. A. D. Peacock, Dept. Natural History, Univ. of St Andrews
London . .	L	Origin not known. Donor, Dr A. G. Hamilton, Dept. Biology, St Thomas Hospital Medical School
Reading . .	R	Sub-cultured from London population, no subsequent mixing. Donor, Dr I. M. Crichton, Dept. Zoology, Reading Univ.
Surbiton . .	S	Origin not known. Donor, Miss E. S. Bartlett, Min. Agric. and Fish., Surbiton

TABLE 2

Incidence of interchange heterozygotes in ten populations of P. americana

Locality	Classes					Total individuals
	16 II	14 II+ 1 IV	12 II+ 2 IV	13 II+ 1 VI	11 II+ 1 VI+ 1 IV	
(*a*) Wild						
P	4	4	2	1	...	11
C	5	8	1	1	...	15
T 1	12	16	10	7	5	50
T 2	12	22	9	...	...	43
(*b*) Culture						
TC	10	21	12	1	6	50
M	3	8	3	3	3	20
D	1	6	5	...	2	14
L	...	5	1	2	1	9
R	5	7	3	...	...	15
S	1	5	4	...	1	11

be higher in nature than in culture (table 4). Furthermore, the average number of interchanges per individual (*i.e.* the coefficient of

TABLE 3

The contingency table for the distribution of karyotypes in wild and laboratory populations

Locality	Classes					Total individuals
	II	1 IV	2 IV	1 VI	1 VI+ 1 IV	
Wild . .	33	50	22	9	5	119
Culture . .	20	52	28	6	13	119
Totals .	53	102	50	15	18	238

$\chi^2_{[4]} = 8{\cdot}1$ $\quad P = 0{\cdot}05-0{\cdot}1$

TABLE 4

The contingency table for the distribution of homozygotes and heterozygotes in wild and laboratory populations

Locality	Classes		Total individuals
	Homozygotes	Heterozygotes	
Wild . . .	33	86	119
Culture . . .	20	99	119
Totals . .	53	185	238

$\chi^2_{[1]} = 4{\cdot}1$ $\quad P = 0{\cdot}02-0{\cdot}05$

TABLE 5

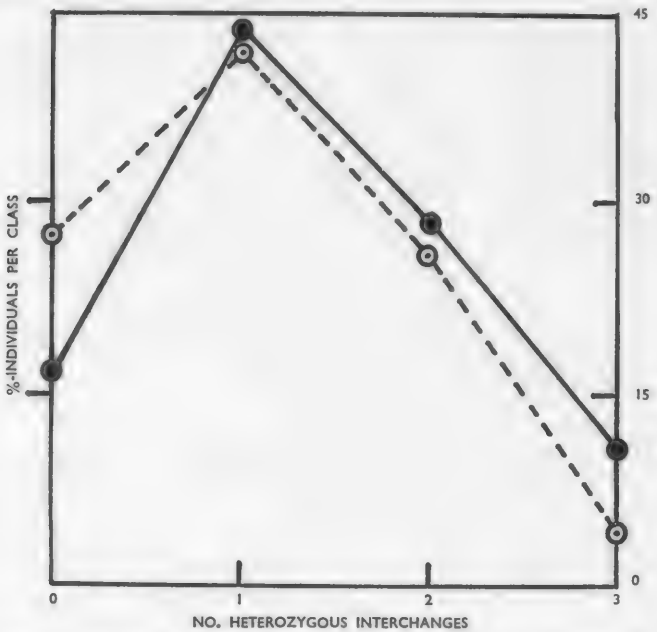
Comparison of the coefficient of interchange heterozygosity in wild and laboratory populations

Locality	Coefficient of I.H.	Standard deviation	Standard error
Wild	1·067	0·841	0·0771
Culture . . .	1·340	0·886	0·0812

$$\frac{\bar{d}}{s_{\bar{d}[237]}} = \frac{0{\cdot}273}{0{\cdot}112} = 2{\cdot}4 \qquad P = 0{\cdot}01-0{\cdot}02$$

interchange heterozygosity) is higher in laboratory cultures than in nature, the variance in both being approximately the same (table 5).

The distribution of individuals with different degrees of heterozygosity is shown in text-fig. 1.



TEXT-FIG. 1.—Frequency of individuals with different levels of heterozygosity in wild (- - -) and laboratory (——) populations of *P. americana*.

TABLE 6

The degree of maximum multiple formation

Karyotype	Per cent. maximum multiple formation								Total individuals	Total cells
	No. of individuals observed per class									
	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100		
1 IV	...	2	...	2	3	14	15	24	60	1863
2 IV	2	1	1	...	2	10	7	7	30	964
1 VI	...	...	...	...	...	...	1	11	12	431
1 VI+1 IV	...	...	...	...	...	1	3	6	10	410

3. FERTILITY

Fertility will depend in part on both the frequency of multiple formation and the orientation of the multiples. The degree of maximum multiple formation is high for all karyotypes (table 6).

As expected, it is lower in individuals heterozygous for two independent interchanges but, contrary to expectation, the ring-of-six heterozygotes appear to have the most regular pairing. Nine out of twelve of these heterozygotes showed 100 per cent. maximum multiple formation. We can suggest no reason for this.

TABLE 7

The occurrence of univalents

Individual no.	Type	Total cells	Univalent type	Times observed	Cause
TC-11	2 IV	40	R IV+ C III+ 12 II+ 1 I	1	Failure of interchange chromosomes
			C IV+ 13 II+ 2 I	1	
D-6	2 IV	12	R IV+ 13 II+ 2 I	1	
			2 IV+ 11 II+ 2 I	4	Autosomal failure
D-8	16 II	10	15 II+2 I	1	

Univalents and numerically uneven multiples which would reduce fertility rarely occur. In addition to the individuals described in table 7, two univalent X chromosomes were observed in twenty-six cells of one individual (D-11) which were at the first division of meiosis.

TABLE 8

Frequency of disjunctional orientation of associations of four

Orientation	Karyotype				Total	Frequency of disjunction
	1 IV		2 IV			
	Rings	Chains	Rings	Chains		
Disjunctional . .	49	1	35	31	116	0·92
Non-disjunctional .	8	...	2	...	10	
Total cells . .	58		46			

Normal chromosome counts were obtained at second division and at mitosis. The aberration, therefore, was not systematic and probably arose by non-disjunction of the X chromosome at a mitosis preceding meiosis by at least five divisions. As in normal cells, the X chromosomes were positively heterochromatic and, due to this precocity, they did not pair (*cf.* Makino, 1939 ; Suomalainen, 1946).

The frequency of disjunction also is high (table 8). For example,

in TC-3, heterozygous for a single interchange, the ring was disjunctional in 0·8 of the cases and in TC-11, heterozygous for two independent interchanges, the multiples in seventeen cells were invariably disjunctional. An association of six has been observed at metaphase in only seven cells but was disjunctional in all of them (text-fig. 2).

TEXT-FIG. 2.—Orientated pro-metaphase with one disjunctional ring-of-six and one disjunctional ring-of-four to the extreme left, X to extreme right. Chromosomes aligned for clarity (TC-7, α-bromonaphthalene 1 hr. ×1800).

4. THE TREHARRIS POPULATION

Our earlier investigation did not reveal the presence of multiple associations higher than four in the wild population from Treharris although they occurred in the laboratory culture. This can now be attributed to sampling error, a possibility we did not exclude. Ring-of-six heterozygotes have been found in T 1 but not, as yet, in T 2. A χ^2 test for heterogeneity showed that the populations in these two localities were significantly different (table 9).

TABLE 9

The contingency table for the distribution of karyotypes in T 1 and T 2

Locality	Classes					Total individuals
	II	1 IV	2 IV	1 VI	1 VI+ 1 IV	
T 1	12	16	10	7	5	50
T 2	12	22	9	...	...	43
Totals	24	38	19	7	5	93

$\chi^2_{[4]} = 12{\cdot}5$ $\quad P = 0{\cdot}01-0{\cdot}02$

At pachytene in *P. americana* the centromere often appears as a constriction and repulsion is localised between homologous centromeres (plate, fig. 5). This makes it possible to determine the point of interchange and, in all cases, this has occurred at or near the centromere. Pachytene associations in many cases can be distinguished by the lengths and arrangement of their arms.

(i) *Associations of four*

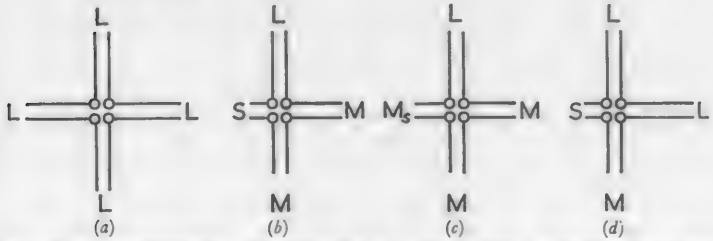
Four morphologically distinguishable cross associations have been observed.

Type 1.—with four long arms of approximately equal length (plate, fig. 1, Lewis and John, 1957 ; text-fig. 3*a*).

Type 2.—with a long, two medium and a short arm in that order (plate, fig. 1, text-fig. 3*b*).

Type 3.—with a long, two medium and a medium-short arm in that order (plate, fig. 2, text-fig. 3*c*).

Type 4.—with two long, a medium and a short arm (plate, fig. 3, text-fig. 3*d*).



TEXT-FIG. 3.—Diagrammatic representation of the morphologically distinguishable pachytene associations of four.

(*a*) IV Type 1 (*cf.* plate, fig. 1, Lewis and John, 1957)
(*b*) IV Type 2 (*cf.* plate, fig. 1)
(*c*) IV Type 3 (*cf.* plate, fig. 2)
(*d*) IV Type 4 (*cf.* plate, fig. 3).

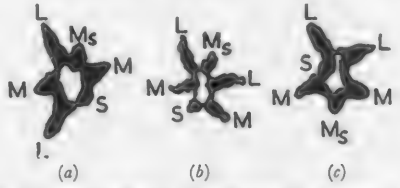
(II) *Associations of six*

Three morphologically distinguishable associations of six have been observed so far. In all cases the pachytene associations are approximately star-shaped indicating short differential segments. Also, all three association types consist of two long, two medium, one medium-short and one short arm. The arrangement of the arms in the three types is as follows :—

Type 1.—with the arm sequence : long, medium, long, medium-short, medium, short (text-fig. 4*a*).

Type 2.—where the arms are arranged long, medium-short, long, medium, short, medium (text-fig. 4*b*), and

Type 3.—with the arms in the following order : long, long, medium, medium-short, medium, short (text-fig. 4*c*).



TEXT-FIG. 4.—Types of morphologically distinguishable pachytene associations of six. (× 1250).

(*a*) VI Type 1 (*cf.* plate, fig. 6, Lewis and John, 1957)
(*b*) VI Type 2 (*cf.* text-fig. 5)
(*c*) VI Type 3.

The following observations are also relevant to the explanation which we advance in the next section to account for these configurations :

(i) Ring IV Type 3 and Ring VI Type 2 have been found in the same individual (text-fig. 5).

(ii) Karyotypes heterozygous for three independent interchanges were recorded in our earlier investigation.

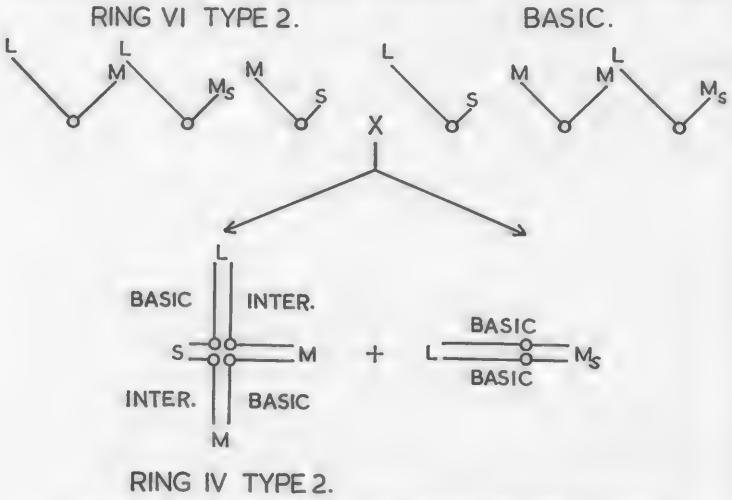
(iii) Rings higher than six have never been observed, and

(iv) Individuals with two rings-of-six have not been found although in all, over two hundred and fifty individuals have been examined.

TEXT-FIG. 5.—Pachytene with an association of six (Type 2) and an association of four (Type 3). Centromeric regions forming clear gaps (T 1-42, colchicine 2½ hr. × 1250.)

(iii) *The interchanges*

The number of interchanges necessary to account for the above configurations is decreased if the rings-of-six can give rise to rings-of-

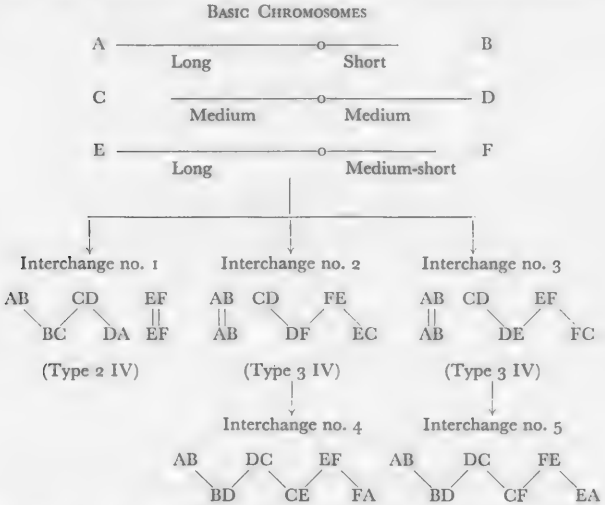


TEXT-FIG. 6.—Derivation of Ring IV Type 2 by crossing Ring VI Type 2 with a basic homozygote.

four when crossed with the basic karyotype. Ring VI Type 2 gives two types of gamete both with (Long : Medium), (Long : Medium-short), and (Medium : Short) chromosomes. This heterozygote can give the Ring IV Type 2 configuration if the basic chromosomes are (L : S), (M : M), (L : Ms) as shown in text-fig. 6.

In the same way the Ring VI Type 3 configuration can give the Ring IV Type 3 configuration but again only if the basic chromosomes are (L : S), (M : M), (L : Ms).

This, together with observations (iii) and (iv) above, have led us to conclude that all the rings-of-six involve the same chromosomes. The apparent absence of individuals heterozygous for more than four interchanges may be because they exceed the hybridity optimum (Darlington and La Cour, 1950). But the coincidence of arm lengths in the observed heterozygotes has led us to the following scheme :



The Ring VI Type 1 can then be obtained by crossing interchange heterozygotes 1 and 3,
Ring VI Type 2 by crossing interchanges 1 and 4,
Ring VI Type 3 by crossing interchanges 2 and 5, and
Ring IV Type 4 by crossing interchanges 3 and 5.

If this peirastic scheme is correct, the various types of configurations, both of four and of six, are mutually exclusive. It would follow, therefore, that the rings-of-four in the VI+IV individuals must be due to other, independent, interchanges : interchanges of two kinds.

Firstly, an interchange (No. 6) involving isobrachial chromosomes to give the Ring IV Type 1 which cannot be derived from any of the rings-of-six. Secondly, an interchange (No. 7) giving a pachytene association of four morphologically similar to the Ring IV Type 3. This is necessary because such a multiple has been found together

with a ring-of-six in the same individual (text-fig. 5). That two morphologically similar associations of Ring IV Type 3 do occur is shown by their occurrence in the same cell, albeit in an individual from another population (C-1).

This explanation on the basis of seven interchanges satisfies all the conditions (i)-(iv) above. However, it also makes possible the formation of pachytene associations of a kind not yet observed. Since analyses of pachytene can be made only infrequently this is not surprising. A more serious objection is that it makes possible the formation of rings-of-six in the T 2 population.

The recorded distribution of morphologically distinguishable interchange associations is given in table 10. To account for this distribution, T 2 need have only interchanges Nos. 1, 3 and 5 while interchange No. 3 may be absent from T 1. However, we do not regard these findings complete. Ring VI Type 1, for example, is expected to occur in T 2 although no rings-of-six have so far been recorded in this population.

TABLE 10

Distribution of morphologically distinguishable interchanges in nine populations of P. americana

Locality	IV				VI		
	Type 1	Type 2	Type 3	Type 4	Type 1	Type 2	Type 3
T 1	+	+	+	...	...	+	+
T 2	...	+	+	+	...	...	...
T C	+	+	+	+	+	...	...
P	...	...	+	...	...	...	...
C	...	...	+	...	...	...	...
M	+	...	+	...	...	...	...
D	...	+	...	...	...	...	...
R	...	+	+	...	...	...	...
S	+	...	...	+	...	...	...

In our earlier account we suggested that the rings now fixed in the heterozygous condition were of a kind normally floating in an outbreeding population. Seven interchanges is by no means unprecedented. Ten different interchanges were recorded in eleven interchange heterozygotes found among 933 individuals of the prosobranch mollusc *Purpura lapillus* (Staiger, 1955).

5. SELECTION FOR HETEROZYGOSITY

Spontaneous chromosome mutations, such as interchanges, occur less frequently than gene mutations. When they arise, they usually remain permanently floating in the population. They are rarely incorporated into the genetic systems of wild animal populations in the heterozygous condition as they have been in many plants, for example, *Pæonia californica*. There appear to be two reasons for this:

Firstly, interchange hybrids, as a result of non-disjunction, may

be semi-sterile and therefore subject to negative selection. This objection does not apply to the cockroach populations. The high frequency of multiple formation and their regular disjunction indicate high fertility. This is expected for, being under genetic control, both these chromosome characters are subject to selection (Rees, 1955 ; Thompson, 1956 ; Lawrence, 1958). Although selection will operate after the occurrence of interchanges, *Periplaneta*, like *Œnothera* and *Rhœo*, is pre-adapted in its complete terminalisation. In addition, its meiosis is characterised by a pre-metaphase stretch which, so we believe, further facilitates disjunction (Lewis and John, 1957).

Inbreeding a normally outbreeding species results in increasing homozygosity and consequent unbalance. Interchanges, by creating differential segments and by extending the linkage group, can preserve heterozygosity. In animals, where the sexes are separate, this change in the breeding system is less common and is a second reason why interchange heterozygosity is rarely established.

This objection again does not apply to the present populations. All are inbred. *P. americana* is not native to this country and we have never encountered populations on the surface (Lewis and John, 1957). The wild coal mine populations are therefore comparable with isolated communities of a species at the limit of its range : communities similar to those in *Pæonia* and *Œnothera* where interchange hybridity has been established (Darlington, 1956). Of course, in the case of *P. americana* in this country the range cannot extend.

The laboratory cultures are also inbred because they are continually decreased in size. Indeed, the higher coefficient of interchange heterozygosity would suggest that they are even more inbred than the wild populations. This is probably true for they are usually depleted in every generation and closer inbreeding will follow such a decrease in population size.

The populations from the two localities within the coal mine at Treharris were different. Although these are separated by less than a few hundred yards this probably means that they are spatially isolated. The only roadway connecting the populations is permanently illuminated and in constant use ; these factors probably discourage migration.

It was suggested that five of the seven interchanges in the Treharris material had occurred in three chromosomes. This means that there is a higher probability of producing individuals heterozygous with respect of these chromosomes in the same way as multiple alleles facilitate genic heterozygosity. It has been shown in rye that selection does not favour heterozygotes indiscriminately but rather favours heterozygosity in particular chromosomes (Rees, 1956). If this is the case in *P. americana* it would appear that heterozygosity in these three chromosomes is favoured in particular for there is no reason to believe that interchanges arise more often in some chromosomes than in others.

The pachytene associations observed in other populations are morphologically similar to those in Treharris. Crosses are now in progress to determine whether these are dissimilar interchanges or similar interchanges of dissimilar ancestry.

These investigations on *P. americana* show how inbreeding can result in the establishment of structural hybridity even in a dioecious species. They also show that different populations can evolve independently in the same direction as have different sections of the genus *Œnothera*. Indeed our unpublished investigations on cockroaches of different genera suggest that this may apply to larger taxonomic groups.

On the other hand, unrelated groups often have propensities for chromosome change in different directions, for interchange heterozygosity is not the only way of maintaining balance on inbreeding. In *Locusta migratoria* we would suppose that supernumerary chromosomes serve this purpose because they have been found in all the inbred laboratory cultures examined (Rees, 1954). This may be true also of *Cimex* species where the frequency of supernumerary " X " chromosomes is different in culture than in nature (Darlington, 1939). In this connection it is significant that in *Œnothera* the addition of different extra chromosomes can render a particular lethal diploid combination viable (Catcheside, 1936). In *Drosophila* on the other hand inversions have been exploited. Perhaps the direction of change is determined by pre-adaptation. On this basis the establishment of interchange heterozygosity, for example, is unlikely in a species like *Allium fistulosum* which has proximally localised chiasmata and no terminalisation.

Although there are many ways in which the chromosome mechanism can resist changes in the breeding system most of the permanent diploid hybrids in nature are interchange heterozygotes. This is probably because only interchanges can join determinants on different chromosomes together in the same linkage group.

6. SUMMARY

1. Four wild populations and six laboratory cultures of *P. americana* are described which have a high frequency of interchange heterozygotes. All these populations are inbred.

2. The multiples form and disjoin with a regularity expected to give a high fertility. It is suggested that *P. americana* is pre-adapted for the establishment of interchange heterozygosity in :

(i) Complete terminalisation, and

(ii) The presence of a pre-metaphase stretch at meiosis, both of which facilitate disjunction.

3. Interchange heterozygosity has been established independently in a number of populations. Interchanges arise infrequently and their establishment in the heterozygous condition must be due to selection.

Plate

FIG. 1.—Type 2 pachytene cross of four (T2-1, colchicine 6½ hr. ×4000).

FIG. 2.—Type 3 pachytene cross of four (TC-11, untreated. ×4000).

FIG. 3.—Type 4 pachytene cross of four (T2-5, colchicine 7 hr. ×4000).

FIG. 4.—Induced "diakinesis" in a homozygote (R-4, colchicine 1 hr. ×1250).

FIG. 5.—Pachytene in a ring-of-four heterozygote (S-6, colchicine 1½ hr. ×2000).

FIG. 6.—Induced "diakinesis" in an individual heterozygous for two rings-of-four (M-7, colchicine, 3½ hr. ×2000).

FIG. 7.—Induced "diakinesis" in a ring-of-six heterozygote (M-6, colchicine 4 hr. ×2000).

FIG. 8.—Induced "diakinesis" in an individual heterozygous for a ring-of-six and a ring-of-four (M-5, colchicine 3 hr. ×2000).

FIG. 9.—First metaphase in a chain four heterozygote. Chain in disjunctional orientation (TC-11, untreated. ×4000).

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4. It can be correlated with a change in the breeding system from outbreeding to inbreeding as has been shown in *Campanula* and inferred in *Œnothera*.

5. To account for the number and morphology of the multiples, seven interchanges are indicated in the Treharris material, five of these in three homologous pairs. This indicates that selection favours heterozygosity for these chromosomes.

6. It is suggested that related groups may show a propensity for chromosome change in the same direction determined, perhaps, by pre-adaptation.

7. Of the chromosome mechanisms which can resist changes in the breeding system interchange heterozygosity is favoured because this alone extends the linkage group to cover more than one chromosome.

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GENETICAL STUDIES IN ACACIAS

II. LEAF CHARACTERS IN HYBRIDS BETWEEN BLACK AND GREEN WATTLES

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1. INTRODUCTION

THE investigations described in this paper follow directly on those of Philp and Sherry (1949) who, in 1943, made what was probably the first attempt to produce hybrids between black wattle (*Acacia mollissima*) and green wattle (*A. decurrens*) under controlled conditions. As a result of these cross-pollinations, many hybrid seedlings were obtained from two green wattle trees used as seed parents. They described in detail the methods of hybridisation and also the leaf characters of the parent trees of the F_1 hybrids, and of an open-pollinated family (presumed to be an F_2) from a naturally occurring hybrid.

By 1948, when wattle genetics was taken over by the Wattle Research Institute, the F_1 hybrids were flowering freely and in the following years, F_2 and back-crossed families were raised. The study of the characters, which appeared to be controlled by polygenes, was continued with a view to determining their behaviour in interspecific crosses. If information could be obtained on the segregation and recombination of leaf characters which are easily measurable, it could be argued that other characters, also controlled by polygenes, but more difficult to determine precisely, would behave in a similar manner and some indication of the possibilities of combining such characters in interspecific hybrids would be obtained.

2. MATERIAL AND METHODS

One of the two green wattle trees used by Philp and Sherry as seed parents, G33, was crossed with an unrecorded black wattle tree; the other, G35, with a tree numbered B18. There were a number of seedlings in the F_1 from G33, but only two survived and it was possible to self only one of these. Description has, therefore, been confined largely to five sister families from G35×B18. The two F_1 families from the above crosses were numbered H33/44 and H35/44 respectively. In H35/44, nine trees survived, but further work was restricted to five trees, namely H35/44/19, H35/44/21, H35/44/24, H35/44/25 and H35/44/30. For brevity these trees are referred to as H33/24 (from family H33/44) and H35/19, H35/24 etc. (from family H35/44).

The yield of seed from these trees was low, more especially following selfing and back-crossing to the green parent, and seed had to be saved from three seasons' bagging (1949-51) before sufficient could be accumulated to give reasonably-sized families.

Samples of leaves were taken when the F_2 and back-crossed families were $3\frac{1}{2}$ years old, at which age they had largely assumed their mature foliage. Six

leaves were taken at random from each tree with the limitation that, as far as possible, leaves from similar positions on the branch and of the same degree of maturity, were chosen. Leaves were pressed in the field and measurements made on the dried specimens in the laboratory.

The characters measured were : rachis length, rachilla length, pairs of pinnæ, and pinnule number, spacing, and length. Pinnule spacing was calculated by measuring the length of a pinna central on the leaf and dividing it by the number of pinnules on it. This measurement gives results which differ somewhat from those of Philp and Sherry, who used direct measurement of the spaces, and it has the disadvantage of introducing a ratio into some of the correlations. It has, however, proved to be an efficient way of scoring spacing and involves very much less labour than direct measurement. Measurements made on a central pinna were found to give a very good approximation to the average of all pinnæ on the leaf.

There were significant differences between the species for all the characters measured but, for simplicity, description has been confined to the last four, since these give a good picture of the salient differences in leaf form between the species.

3. FERTILITY OF THE F_1 HYBRIDS

Seed was collected from the F_1 hybrids following four different types of pollination, three of which, selfing and back-crossing to the two parents, were controlled by bagging. With the fourth type—open-pollination—there was a possibility of pollination by selfing, intercrossing between the F_1 trees, or natural back-crossing to the green and black parents. Because of the position of the trees, there was every opportunity for the F_1 hybrids to cross with green wattle, but much less possibility of pollination by black wattle.

Fertility was assessed first by the number of seeds set per bag and secondly by the mean number of seeds per pod. Fertility following different pollinations is summarised in table 1.

For the back-crosses, the F_1 trees were used as seed parents, and since emasculation is not practicable, selfed as well as back-crossed seeds may have been produced. It should be mentioned here that in both the F_2 families and in the back-crosses to green wattle, a considerable proportion of chlorotic seedlings or seedlings which were chimæras of chlorotic and normal tissues, appeared (Anon., 1952). Leaf measurements in the families from back-crosses to green showed subsequently that many of the seedlings were, in fact, due to self-pollination.

Following pollination with black wattle, on the other hand, there was a comparatively high seed-set in the F_1 hybrids and only a very small proportion of chlorotic seedlings occurred. It was concluded that the great majority of seedlings in these families were back-crosses and this conclusion was confirmed later by leaf measurements. It would seem, therefore, that the F_1 hybrids are much more fertile with the black than with the green wattle parent. The low fertility with the latter probably accounts for the comparatively low seed-set following open-pollination since, as has been mentioned, there was little likelihood of natural back-crossing to black wattle, but ample opportunity for crossing with green.

4. CYTOLOGY OF THE F_1 HYBRIDS

Since the hybrids showed considerably reduced fertility compared with the parents and produced abnormal types in the F_2, it seemed possible that they might show chromosome irregularities at meiosis. Both parents and hybrids had the chromosome number $2n = 26$. At meiosis in the hybrids, 13 bivalents were formed with great regularity and in no instance was any chromosome abnormality observed. It was not possible to determine whether there was any reduction on chiasma frequency compared with the parents. In

TABLE 1

F_1 hybrids. Total seeds collected and mean per bag and per pod following different pollinations

	Selfed			× Green wattle			× Black wattle			Open-pollination	
	Total seeds	Mean per bag	Mean per pod	Total seeds	Mean per bag	Mean per pod	Total seeds	Mean per bag	Mean per pod	Total seeds	Mean per pod
H33/24	18 (3)	6·0	3·0	...	...	...	...	...	...	143	3·6
H35/19	119 (16)	7·4	1·0	35 (2)	17·5	2·3	130 (2)	65·0	3·8	40	1·7
H35/21	158 (15)	10·5	0·8	117 (8)	14·6	2·2	594 (5)	118·8	3·7	103	2·6
H35/24	91 (6)	15·2	0·6	...	...	...	...	...	...	139	3·5
H35/25	114 (16)	7·1	0·7	40 (2)	20·0	1·5	233 (2)	116·5	4·7	113	2·8
H35/30	15 (2)	7·5	0·9	...	...	...	...	...	...	...	...
Mean		8·9	1·2		16·0	2·0		106·3	4·0		2·8

Figures in brackets give the number of inflorescences on which the bags were undamaged.

view of the regular chromosome pairing and separation in the hybrids, it must be assumed that reduced fertility and abnormal seedlings in the F_2 are due to genic unbalance and not to cytological disturbances.

5. LEAF CHARACTERS IN THE PARENT SPECIES

Leaf characters on any one tree fluctuate with varying environmental conditions and with the age of the tree, so that comparisons beween trees of different ages or on different sites are liable to considerable errors. Young trees tend to have comparatively large leaves with long, widely-spaced pinnules, whereas older trees have smaller, more compact leaves with shorter, closer-spaced pinnules and a lower pinnæ number. Philp and Sherry's measurements of the leaves of the original parent trees were made when the trees were ten years old and these measurements differed somewhat from the means of younger families from bulk seed and from those of open-pollinated progeny of single trees. For further comparisons during 1955, leaf measurements were made of 45 black wattle and 24 green

wattle trees (table 3) of approximately the same age and growing under similar conditions as the F_2 and back-crossed families. These groups gave somewhat different measurements from the parent trees, but the group means probably provide a better standard for comparison for the hybrid families than do the parent trees. Measurements on E_5 and B_5, the respective parents of the back-crosses, were also made when the trees were considerably older than the hybrid families and these also tend to give a somewhat distorted comparison. It should be noted, however, that while age and environment cause fluctuations in the characters, the differences between the species are

TABLE 2

Mean leaf measurements and standard errors (single leaf) of parent trees and F_1 hybrids

Parent trees and F_1 hybrids	Pairs of pinnæ	Number of pinnules	Length of pinnules in mm.	Pinnule spacing in mm.
Green wattle G35 .	7·3±1·13	...	9·6±0·78	...
Black wattle B18 .	16·3±1·62	...	3·3±0·25	...
Green wattle E5 .	7·0±0·42	27·0±4·70	8·8±1·25	1·76±0·24
Black wattle B5 .	16·2±2·07	49·5±4·70	3·0±0·15	0·85±0·05
F_1 hybrid H35/19 .	12·4±1·56	38·3±4·06	4·6±0·48	1·13±0·10
F_1 hybrid H35/21 .	11·2±0·99	34·2±2·45	4·5±0·39	1·27±0·10
F_1 hybrid H35/24 .	13·8±0·85	39·4±2·26	4·8±0·42	1·02±0·10
F_1 hybrid H35/25 .	12·6±1·56	39·2±5·90	4·7±0·46	1·10±0·09
F_1 hybrid H35/30 .	11·8±1·39	40·9±2·60	4·7±0·56	1·12±0·12

so great for most characters that variation within each species does not seriously affect the results. Leaf measurements for parents and hybrids are shown in table 2.

The species differ significantly in their means for each character and there is great variation within each species, trees differing significantly from each other for each of the characters (Philp and Sherry, *loc. cit.*). The shape of the frequency curves and the continuous variation within each species pointed to control by polygenes, while segregation gave trees having different combinations of these genes and hence different degrees of expression of the characters. For instance, both species show significant intraspecific variation for pinnæ number, but the ranges of the two species do not overlap. In both species, gametes differing in the number of genes for this character will be formed and, depending upon the assortment of these gametes, trees differing in pinnæ number may be expected in the F_1 hybrids. This expectation was fulfilled, for although the F_1 trees were approximately midway between the parents for all four characters, significant differences between sister F_1 hybrids occurred for all characters except pinnule length, and these differences persisted in later generations.

6. F_2 AND BACK-CROSSED FAMILIES

There was a wide range of variation in all four characters within the F_2 and, in some families, leaf types occurred which came close to black wattle at one end of the scale and to green wattle at the

TABLE 3

Mean leaf measurements and standard errors (single tree) of F_2, back-crossed families, and bulk black and green samples

Family	No. of trees	Derived from	Pairs of pinnæ	No. of pinnules	Length of pinnules mm.	Pinnule spacing mm.
H12/51	61	H35/19 s.	13·6±1·8	40·1±5·1	4·57±0·85	1·14±0·16
H13/51	100	H35/21 s.	11·9±1·4	39·4±5·1	4·87±0·79	1·20±0·16
H14/51	39	H35/24 s.	15·5±2·3	43·9±7·2	4·34±0·60	1·03±0·12
H15/51	41	H35/25 s.	15·2±1·9	41·6±5·5	4·83±0·54	1·19±0·13
H16/51	13	H35/30 s.	14·7±1·7	40·4±5·8	4·37±0·41	1·08±0·09
Diff. sig.			**	**	**	**
H17/51	43	H35/19×B5	17·5±1·7	50·7±6·4	3·50±0·34	0·96±0·10
H18/51	34	H35/21×B5	16·5±1·8	46·2±6·6	3·66±0·49	0·97±0·10
H19/51	32	H35/25×B5	18·4±3·0	48·6±6·8	3·51±0·38	0·93±0·10
Diff. sig.			**	**		
H7/52	22	H35/21×E5	10·3±1·0	34·1±4·6	7·36±1·03	1·57±0·19
H8/52	9	H35/25×E5	11·3±1·0	33·1±3·4	7·52±0·70	1·59±0·10
H9/52	6	H35/19×E5	10·8±0·6	38·2±3·4	6·91±0·78	1·47±0·17
Diff. sig.			*			
H11/51	36	H33/24 s.	11·9±1·7	33·8±6·3	4·29±0·90	1·16±0·18
Green	24	Bulk seed	9·3±0·5	35·5±3·3	7·95±0·86	1·72±0·13
Black	45	Bulk seed	17·6±1·7	47·5±5·3	3·01±0·34	0·95±0·09
Diff. sig.			**	**	**	**

s. = selfed.

* Significant at 5 per cent. level. ** Significant at 1 per cent. level.

other. The range of variation, however, did not extend over the same limits in each family and there were significant differences between the means for all four characters (table 3).

The scatter diagrams in figs. 1 and 2 show the range covered by each of the four characters in two F_2 families, H13/51 and 14/51, derived respectively from H35/21, the F_1 tree closest to green wattle, and H35/24, that nearest to black wattle. The means of the parent species and of the F_1 trees are shown by crosses on the diagrams, the

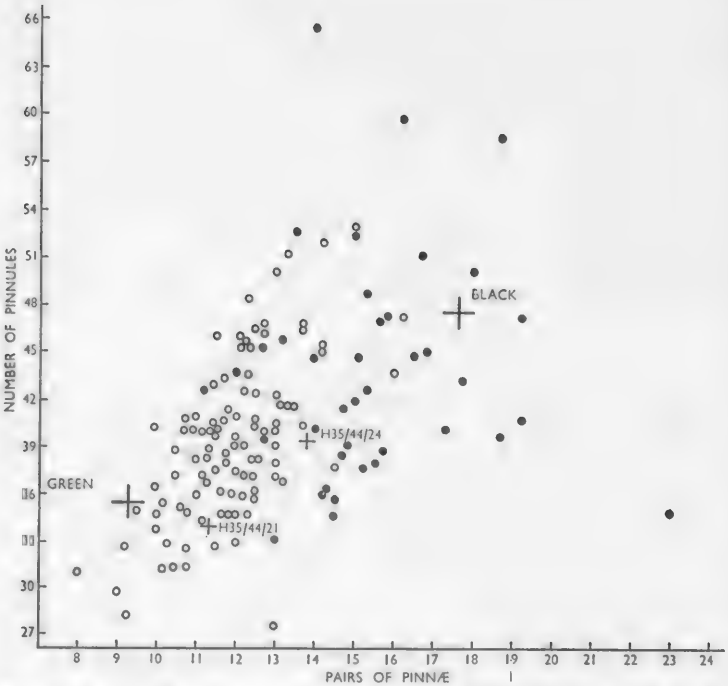

FIG. 1.—Segregation and correlation of pinnule number and pinnæ number in two F_2 families from G35×B18. Open circles, H13/51, the F_2 from the F_1 tree having the lowest value for the two characters (H35/44/21 = H35/21) and black circles, H14/51, the F_2 from the F_1 tree having the highest value for these characters (H35/44/24 = H35/24). In this and the following figures the characters in the parent tree or parent species are shown by vertical and horizontal arms of the crosses.

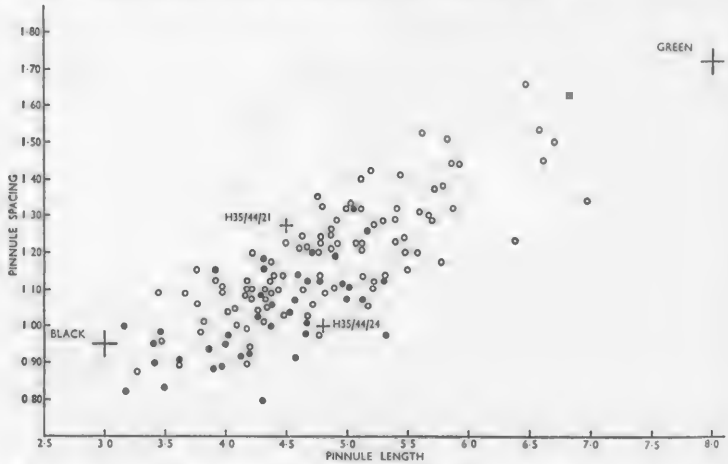

FIG. 2.—Segregation and correlation of pinnule length and pinnule spacing in same two families as fig. 1. Open circles H13/51 ; black circles H14/51.

vertical axis giving the mean for one character and the horizontal axis the mean for the other.

Except, possibly, for pinnule length, the mean expression of a character in the F_2 is clearly correlated with the degree of expression of that character in the F_1 parent. In back-crosses to the parent species, a similar correlation occurred. Three of the F_1 trees were crossed to a single tree of green wattle (E5) and to a single tree of black wattle (B5). The variation in families from two of these F_1 trees, H35/21 having the lowest pinnule and pinnæ number and H35/25 having higher numbers, is shown in figs. 3 and 4 respectively. A comparison of the two graphs shows that when the F_1 tree having the higher value for the two characters is used as parent, the means of the F_2 and both back-crosses derived from it are higher than when the F_1 parent with the lower pinnæ number is used. Apart from pinnule and pinnæ number, this parent-progeny correlation affects the overall appearance of the leaf as shown in pl. I. H35/21 has a leaf-shape rather akin to that of green wattle while H35/25 resembles black in general shape. The photographs show that the leaf-shape of particular F_1 parent trees persists to a considerable degree in both F_2 and back-crossed families.

In the back-crosses to green wattle, although the pinnule number approached or overlapped that of E5, from neither cross did the pinnæ number reach that of the green parent. In the back-crosses to B5, on the other hand, not only was there considerable overlapping of the black parent for both characters, but in the back-crossed progeny from H35/25, individuals occurred which had a higher number of pairs of pinnæ than we have so far recorded in black wattle.

7. CORRELATION BETWEEN CHARACTERS

In the F_2 and back-crossed families, it was evident that recombination of leaf characters was not at random, but that there was a tendency for parental types to remain associated. Partial correlations were calculated for each pair of characters in the parents, the F_1, F_2 and back-crossed families to determine the degree of this association (table 4, figs. 3 and 4).

In black wattle, pinnule spacing and pinnule length and pinnule spacing and number of pinnules, showed significant correlation ($P = 0{\cdot}01$), while in green wattle, pinnule spacing and pinnule length were correlated ($P = 0{\cdot}05$). The correlations in the parent species were surprising and may possibly be explained by the fact that pinnule spacing, pinnule length and number of pinnules on an individual tree are influenced by environmental conditions. For instance, shade leaves tend to have fewer, but longer and more widely spaced pinnules than leaves exposed to full sunlight. These differences within species are not great compared with differences between species, but any parallel variation in the three characters, however small, will show up as a correlation. In the hybrid families, the expression of these

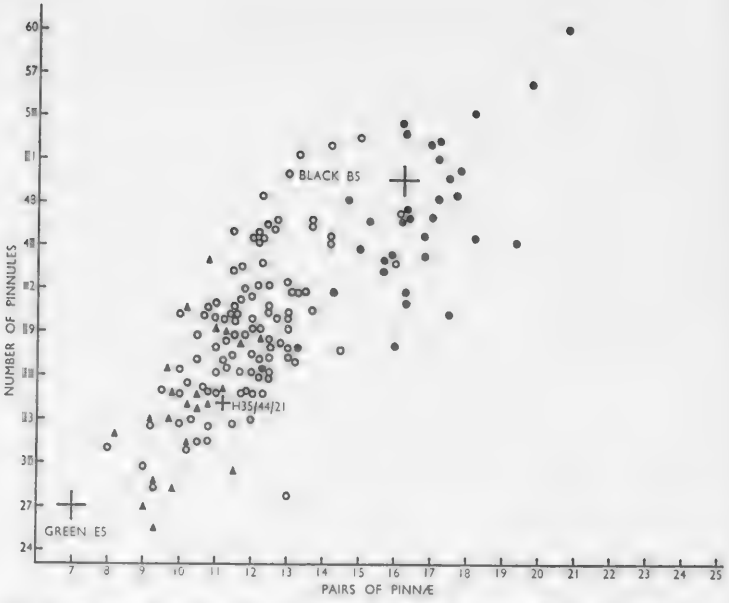

FIG. 3.—Pinnule number and pinnæ number in the F_2 and back-crosses from H35/44/21 (= H35/21), the F_1 tree having the lowest value for these characters. Open circles H35/21 selfed (H13/51), triangles H35/21 crossed to green wattle tree E5 and black circles H35/21 crossed to black wattle tree B5.

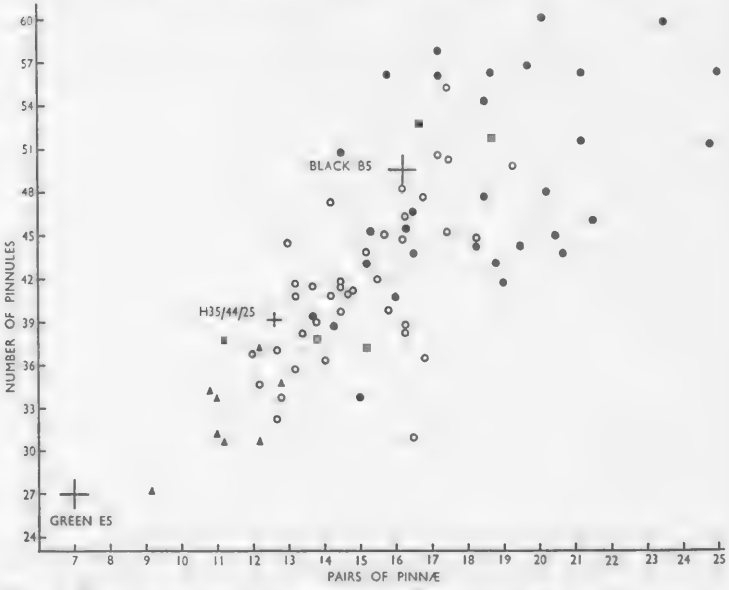

FIG. 4.—Pinnule number and pinnæ number in the F_2 and back-crosses from H35/44/25 (= H35/25), an F_1 tree having a high value for the two characters. Open circles, H35/25 selfed (H15/51), triangles H35/25 crossed to green wattle tree E5 and black circles H35/25 crossed to black wattle tree B5.

characters was of a different order and there is little doubt that the significant correlations shown in table 4 are due, in part at least, to the association of genes which determine the characters.

The number of individuals in the F_1 was too low to determine with any degree of certainty whether there was any significant correlation between the characters. There was, however, some indication that pairs of characters tend to be associated. Taking again two of the most divergent F_1 trees, H35/21 and H35/25, the former having the lower number of pairs of pinnæ also had a lower number of pinnules, and wider spacing between the pinnules than the latter (table 2, plate I). Correlation of characters in the F_1, if it really occurs, would point to a pleiotropic effect of the genes concerned.

In the F_2 families, all combinations of pairs of characters showed correlation to a greater or lesser extent, that between pinnule spacing and pairs of pinnæ being weak and showing significance only when the five F_2 families were grouped.

Tests were made for each pair of characters to determine whether there were significant differences in the correlation coefficients within each group of families derived from the H35 hybrids For the most part the differences were well within the limits of sampling errors, but in the F_2 group, for two pairs of characters the correlations differed significantly between the families, those between pinnule spacing and pinnule length at the 5 per cent. level and between pinnule number and pairs of pinnæ at the 1 per cent. level (table 4). Since correlations between parental characters in segregating generations may be due to pleiotropy, gametic and zygotic elimination, or linkage, it must be assumed that the five F_1 trees, from which these F_2 families were derived, differ amongst themselves for one or more of these factors. The correlations can hardly be due to pleiotropy since, if they were, they would be expected to be consistent and not to show abrupt changes such as occur in the F_2. Gametic and zygotic elimination may account for some of the overall correlation of characters, but it seems unlikely that they are responsible for the heterogeneity in the F_2 families. If they were the cause, it is improbable that only one out of the six pairs of characters would be affected, as is clearly the case in H14/51, and it is likely that differences in the fertility of the F_1 trees on selfing would occur. As shown in table 1, there is no indication of any such differences. It seems probable, therefore, that the heterogeneity of the correlations is due to changes in the linkage relations of the characters concerned.

There was no evidence of heterogeneity in the back-crossed families, except possibly in the back-cross to black wattle, where the coefficient between pinnule number and pairs of pinnæ approached the level of significance. When the families were grouped within each back-cross, the correlation coefficients were very much of the same order as the F_2, taking into account the smaller number of individuals involved.

TABLE 4

Partial correlations between pairs of characters in parent species, F_1, F_2, and back-crossed families

A = pinnule spacing ; B = pinnule length ; C = number of pinnules ; D = pairs of pinnæ

Family	Number of trees	D.F.	r AB CD	r AC BD	r AD BC	r BC AD	r BD AC	r CD AB
Black	45	41	+0·615**	−0·430**	−0·154	+0·083	+0·044	+0·091
Green	24	20	+0·498**	−0·329	−0·221	+0·201	+0·068	+0·115
F_1	5	1	+0·484	−0·165	−0·052	+0·645	−0·688	+0·916
H12/51	61	57	+0·821**	−0·640**	−0·043	+0·402**	−0·275*	+0·422**
H13/51	100	96	+0·791**	−0·487**	+0·222*	+0·346**	−0·376**	+0·643**
H14/51	39	35	+0·723**	−0·681**	+0·210	+0·467**	−0·481**	+0·157
H15/51	41	37	+0·465**	−0·418**	+0·270	+0·295	−0·484**	+0·685**
H16/51	13	9	+0·640*	−0·062	−0·074	+0·191	−0·325	+0·250
χ^2 Heterogeneity	...	4	11·282	6·835	3·748	3·940	1·896	13·393
P	...	...	0·05—0·02	0·20—0·10	0·50—0·30	0·50—0·30	0·80—0·70	< 0·01
Average r	254	229	...	−0·538**	+0·154*	+0·365**	−0·387**	...
H17/51	43	39	+0·613**	−0·474**	+0·011	+0·239	−0·260	+0·322*
H18/51	34	30	+0·726**	−0·201	+0·004	+0·266	−0·296	+0·721**
H19/51	32	28	+0·631**	−0·554**	+0·283	+0·593**	−0·557**	+0·607**
χ^2 Heterogeneity	...	2	0·752	2·750	1·553	3·514	2·234	5·703
P	...	...	0·70—0·50	0·30—0·20	0·50—0·30	0·20—0·10	0·50—0·30	0·10—0·05
Average r	109	94	+0·656**	−0·423**	+0·088	+0·363**	−0·364**	+0·549**
H7/52	22	18	+0·835**	−0·788**	−0·105	+0·773**	−0·268	+0·297
H8/52	9	5	+0·381	−0·259	−0·495	+0·506	−0·182	+0·421
H9/52	6	2	+0·513	−0·879	+0·351	+0·191	+0·117	+0·586
χ^2 Heterogeneity	...	2	2·288	2·265	0·913	1·256	0·164	0·182
P	...	...	0·20—0·10	0·20—0·10	0·70—0·50	0·70—0·50	0·95—0·90	0·95—0·90
Average r	57	22	+0·773**	−0·733**	−0·162	+0·718**	−0·236	+0·336

* Significant at 5 per cent. level. ** Significant at 1 per cent. level.

8. DISCUSSION

Although green and black wattles may be crossed without much difficulty, the hybrids show considerable sterility compared with the parents. Since meiosis is regular, this sterility cannot be due to gross cytological abnormalities, but must be attributed rather to gene differentiation between the species, leading to genetic or zygotic elimination or to weakly offspring. Gametic elimination almost certainly occurs, but was not easy to assess since pollen is shed in the form of pollinia, each consisting of sixteen grains. There was, however, considerable evidence of zygotic elimination, since failure occurred at all stages from tiny embryos to full-sized, but shrivelled and inviable seeds. Such elimination must necessarily restrict recombination of parental characters and it is not unreasonable to suppose that zygotes having potentially the greatest genetic diversity are the most likely to be eliminated. The F_1 hybrids from G35 were, on the whole, well below the average vigour of the parents and they showed chlorosis in the early stages of growth (Philp and Sherry, *loc. cit.*). These symptoms of unbalance were accentuated in the F_2, where many abnormal and semi-lethal types occurred (Anon., 1952). The individuals on which the leaf characters were measured must, therefore, be considered to be the more balanced residue remaining after the more extreme types had been eliminated.

Most of the differences between the species showed blending inheritance and may be assumed to be controlled by polygenes, but at least one major gene difference occurred also. The difference in the period taken to ripen seed, four months in green wattle and fourteen months in black wattle, appears to be governed by two major genes, either or both of which, when dominant, causes the seed to ripen in the shorter time (Anon., 1955). Leaf characters, however, were all polygenic in inheritance and their behaviour is of considerable interest, since characters of economic importance are likely to behave in a similar manner.

Anderson (1939) has studied in detail recombination in species crosses in *Nicotiana*, and the behaviour of the wattle hybrids would seem to be very similar, taking into account the greater heterogeneity of the wattle parents. He showed that recombinations in the F_2 of species hybrids are only an insignificant fraction of the possible recombinations on free assortment and that there is a strong tendency for parental characters to stay together. This tendency is obvious in the leaf characters studied in wattle hybrids, but it is not confined to the leaf characters only, for although our records are not completed, it is evident that F_2 trees having a foliage type close to one or other of the parents also have flower colour and stem ridges close to that parent. This is in line with Anderson's conclusion that " all quantitative characters of an organism are closely linked with one another ". Such linkage of characters is of considerable practical importance.

* Significant at 5 per cent. level.
** Significant at 1 per cent. level.

It must be assumed that, in wattle hybrids, characters of economic importance such as general hardiness and the percentage, colour, and quality of tannin will follow the pattern of the leaf characters and will, in general, tend to be associated with other, possibly undesirable, characters of the parent from which they were derived and that it may be difficult or impossible to separate them. The fact that pinnule number and pinnæ number are fairly closely correlated in three F_2 families and not in the fourth (H14/51) shows, however, that for some characters at least, the association may be broken at an early stage in the breeding programme.

Since segregation occurs in the F_1, it may be possible by choice of parent trees and by selection amongst the hybrids to start off with a relatively advantageous combination of characters, and since there is a parent-progeny correlation between the F_1 parent and its offspring, this advantage will tend to be passed on to later generations. For instance, the F_1 parent of family H14/51 was closer to black wattle in leaf characters than the other F_1 hybrids and the mean of H14/51 was also closer to black than the other F_2 families. Since all quantitative characters in an organism tend to be linked, it is probable that the tannin characteristics of this family will, on the whole, be closer to black wattle than those of a family having leaf characters more akin to the green parent. Even among the small number of F_1 hybrids derived from G35 ×B18, there was a range of genotypes and it must be expected that if the numbers in the F_1 were greater, or different trees from the species used to make crosses, this range would be greatly increased. Thus, the F_1 hybrids from the green wattle tree G33 were, on the whole, as vigorous as open-pollinated seedlings from that tree, whereas the hybrids from G35 were considerably lower in vigour than its open-pollinated progeny (Philp and Sherry, *loc. cit.*). It is evident that only by making a large number of crosses between different trees from the two species, and by growing a considerable number of F_2 families from the resulting hybrids, could any assessment of the range of variation in the hybrids be reached.

Although great diversity may be expected, it is worth while considering from a practical point of view, the economic value of green and black wattle hybrids. Commercially, black wattle is almost the only species grown, but green has certain attributes on the credit side. It is generally considered to be hardier under dry conditions, it produces straighter (but possibly more brittle) poles, it appears to be less susceptible, but not resistant to damage by bagworm (*Kotochalia junodi*), and probably by froghopper (*Bythoscopus cedaranus* and *Lygidolon lævigatum*), and to recover more quickly when attacked. On the debit side, green wattle has thinner bark, sharp stem ridges which render stripping more difficult, and tannin with higher colour values and poorer quality than black wattle. The credit characters of green wattle are rather vague and of a type hard to assess ; they are of the kind it is difficult or impossible to follow

Photographs of typical leaves of green and black wattles, two diverse hybrids from the same parents and the selfed and back-crossed families from these hybrids.

through the welter of hybrid segregation without making elaborate tests. Possibly the most important and most favourable of these characters to pursue would be the partial tolerance to bagworm attack. But because of the preponderance of black wattle grown, the bionomy of the bagworm is largely adjusted to this species and it seems highly probable that, with its high biotic potential, the bagworm would quickly swing over and attack green wattle just as severely as black if the relative amounts of the two species were reversed. Force is lent to this argument by the fact that although bagworm is an insect primarily attacking species of *Acacia*, in recent years it has attacked and defoliated oaks, pines, eucalypts, and other species (Ossowski, 1956), and has established a breeding cycle on oaks (Ossowski, personal communication).

In practice, it will seldom or never be possible to incorporate one or more quantitative characters of one species with those of the other species without affecting other characters also (Anderson, *loc. cit.*). If, for instance, we wish to combine the high quality tannin of the black wattle with the partial bagworm resistance of the green wattle, then we shall probably have to sacrifice some of the quality on one side and some of the resistance on the other. Where the resistance is of doubtful value this is clearly a bad bargain.

It would seem, therefore, that unless some more clear-cut, or at least easily assessed, advantages are discovered in green wattle, it would be unwise to embark on an elaborate programme attempting to combine its somewhat vague advantages with those of black wattle. There is, however, one exception. The shorter time taken to ripen seed in green wattle appears to be controlled by two major dominant genes. As far as we know, this character has no economic value *per se*, but from a breeding point of view it would be of great value to have seed ripening in four months instead of fourteen. The indications are that by a back-crossing technique transfer of this character from green to black wattle will be a relatively easy matter.

9. SUMMARY

1. Hybrids between green and black wattles can be produced relatively easily, but show a considerable reduction in fertility compared with the parents.

2. Four leaf characters selected for detailed study appeared to be polygenic in inheritance, but significant differences between F_1 trees from the same parents occurred for all four characters. These differences tended to persist in the F_2 and back-crossed families, showing a parent-progeny correlation.

3. Recombination of the characters of the two parents does not take place at random, but there is a marked tendency for characters coming in from one species to remain associated. For some characters there are, however, abrupt changes in the correlation value between F_2 families derived from the same original parents.

4. Because of the blending nature of polygenic characters and the tendency for parental characters to remain associated, it is concluded that, unless some desirable character controlled by major genes is detected in green wattle, a large-scale breeding experiment to attempt to combine the qualities of the two species is not justified.

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MUTAGENIC SENSITIVITY OF MATURING GERM CELLS IN THE MALE MOUSE

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1. INTRODUCTION

It had been assumed until recently that *Drosophila* male germ cells showed only two phases of mutational response to irradiation : post-meiotic and pre-meiotic. With successive matings of males given a single dose of radiation, the post-meiotically irradiated stages would be sampled first. These would be followed by a sterile period lasting a few days. By then, pre-meiotically irradiated stages (spermatogonia) would have matured into ripe sperm. This second phase differed from the first in a lower yield of recessive lethals and *no* effects attributable to chromosome breakage or rearrangement.

Lüning (1952) was the first to show that the "sterile" period was due to a high incidence of dominant lethal mutations in sperm which had been irradiated as spermatids. There has been some disagreement, however, as to the precise stage showing this hypersensitivity. Lüning (*loc. cit.*) and Bateman (1956*a* and 1957*b*) held that the hypersensitivity extended from meiosis to late spermatids. Auerbach (1954) and Khishin (1955), on the other hand, claimed that the hypersensitivity was confined to late spermatids.

In the mouse it has similarly been customary to regard male germ cells as showing two distinct responses to mutagens, post- and pre-meiotic, separated in sampling time, following a single dose, by a sterile period, often of two months or more according to dose. I considered that if lower doses were used, permitting fertility to continue right through this period, the sensitivity of the successive stages of spermatogenesis would be revealed.

There was already evidence that matings towards the end of the pre-sterile period showed a greater mutation rate than earlier matings (Brenneke, 1937 ; Hertwig, 1938 ; Kaplan and Lyon, 1953 ; Russell *et al.*, 1954 ; Auerbach and Slizynski, 1956).

2. MATERIAL AND METHODS

F_1 animals were raised from two stocks originating from Dr D. S. Falconer of the Institute of Animal Genetics, Edinburgh, and designated by him as RCL and NF. They had both been selected for large body-size, and in the RCL line in particular this had produced large litters. The cross was made using RCL mothers. The F_1 animals, besides being large and vigorous, mated readily, with few pseudo-pregnancies and large litters. The mean number of ova per ovulation was about 14.

F_1 males, about two months old, had only their hindquarters irradiated, under anæsthesia with Nembutal diluted ten times with saline and injected intraperitoneally at the rate of 0·11 ml. for every 10 gm. body-weight. The testes were kept outside the body wall by the pressure of an elastic band on the abdomen, and for accuracy of dose were kept pressed against a perspex window a fixed distance from the source. The rest of the body was shielded with 1/10 in. of lead. The radiation was from a 300 kV machine with a filter giving a half-value layer of 2·5 mm. of Cu, and given at the rate of about 100 r/minute.

Each male was mated weekly to four F_1 virgin females that were not his sisters and were examined twice daily for vaginal plugs. The females were usually dissected at 12 days for counts of corpora lutea, implantations and post-implantation losses. In one series they were dissected at 10 days, when the distinction between deciduomata and live fœtuses was less marked but still unambiguous, and there was a clearer distinction between deciduomata and later fœtal deaths.

3. NATURE OF DOMINANT LETHALS

Brenneke (1937) showed that the reduced litter sizes from presterile matings were not due to a reduction in the rate of fertilisation, which was normal, but to more or less severe cytological abnormalities in the cleavage divisions of the fertilised eggs, causing early death of the embryos. This death was not always early enough to prevent the embryo from evoking the normal implantation reaction.

Though "dominant lethal" is a term which could be applied with equal justification to any mutation which caused the death of the heterozygote, it has come by usage to be reserved for very early deaths presumed to have arisen through chromosome disturbance of the kind observed by Brenneke. These would not be expected to enable the embryo to survive much beyond the fifth day, when implantation occurs.

From the references in the literature to "phagocytosis" and "resorption" of dead embryos, one might doubt the possibility of estimating, by dissection of a female, all implantations which had died up to the time of dissection, and of determining the stage reached by each embryo at death. I have found no evidence of resorption. Embryos which die at or near the time of implantation are represented by nothing more than a deciduoma (maternal tissue). At 11 days the deciduoma capsularis turns brown and remains in that condition until parturition, during which time these wart-like objects can be seen scattered among the live fœtuses and of a constant size and frequency, independent of the duration of gestation. A less frequent phenomenon is the death of advanced embryos which have reached a recognised stage. If death is recent, the embryo will be stunted and white. Later the fœtus disintegrates into a brown mush, still enclosed in the fœtal membranes, but the placenta persists in size and remains apparently fresh.

The idea of resorption may have arisen from the observation, at nearly full term, of small warts. These are not, however, in the process of being resorbed or phagocytosed: they have been in that condition since the 12th day.

Dead embryos are thus classifiable into three distinct groups.

(*a*) Those failing to induce any implantation reaction. They will contribute to the discrepancy between the number of corpora lutea and number of implantations. This category has been abbreviated to PRE, for " pre-implantation loss ".

(*b*) Embryos represented as deciduomata, which are similar in structure to those induced artificially by chemical or physical stimulation of pseudopregnant uteri. It follows that their presence is no proof that the embryo was alive at the time that the endometrium was stimulated. They have been loosely referred to by Russell *et al.* (1954) as post-implantation deaths. Following that precedent they are referred to as ED for " early post-implantation deaths ".

(*c*) A very heterogeneous group comprising all embryos dying between implantation at 5 days and dissection, usually at 12 days. A fœtus of some kind, however decayed, is always detectable, though dissection may be needed to disclose it. The amnion is always present and also the fœtal placenta which is a fresh, dark red, and appears to be unaffected by the death of the embryo. This category is designated LD, for " late death ".

Russell *et al.* (*loc. cit.*), who would seem to have classified post-implantation losses into two categories equivalent to ED and LD as defined above, referred to the dividing line as death at 10½ days. This is presumably because what I have called ED does not show obvious necrosis until then. Normal implantations of this age have well-marked fœtuses and fœtal membranes, whereas ED's show nothing but a whitish scar in the cavity under the deciduoma capsularis. From this I conclude that the ED embryo has been defunct long before 10½ days and probably since implantation began.

Dominant lethals *sensu stricto* would be expected to fall under categories PRE and ED but not LD. This is corroborated by the observation that the frequency of LD is the same in control and irradiated matings. Even though one might expect *some* dominant lethal gene mutations to kill the embryo at every stage in its development, they did not occur with detectable frequency. That is, their incidence is too low in relation to the non-genetic causes of fœtal death to manifest itself in these experiments.

The number of implantations has been abbreviated to IMP, and of corpora lutea to CL.

4. CONFUSION OF DOMINANT LETHALS WITH OTHER PHENOMENA

It is theoretically possible that recessive lethals might contribute to PRE, ED and LD. However, they could only show in the first generation if the females were already heterozygous for them, in

which case, the male, being of the same stock, would be equally likely to be heterozygous. The control contribution would then be large relative to any increase resulting from radiation.

In addition to dominant lethals, the class PRE will contain unfertilised eggs, infertile eggs, eggs fertilised by sperm unable to participate in cleavage (at least a theoretical possibility) and fully normal fertilised eggs which are unable to implant owing to maternal failure of the uterus to respond to the usual stimulus. There is also the possibility of copulation which was ill-timed in relation to œstrous, which might result in any of the above defects. The most extreme case of this type of failure is pseudopregnancy (abbreviated to PSP), when none of the eggs becomes implanted. This is distinguishable from complete failure of the mated female to respond to the mating (which occurs occasionally), by dissection at 12 days, when in PSP the CL formed at mating are found to have persisted as large white bodies. Dissection then coincides with the first œstrous following the mating.

I have also found in this material 12-day uteri in which one horn is normal but the other is empty, though the ovary on that side carries the normal crop of CL. I have called this semi-PSP, as one horn has behaved in the same way as the whole uterus in PSP. In such cases the normal horn is evidence that the failure of the other cannot be due to failure of the sperm or timing of insemination, but must arise from some asymmetry of maternal function. Full PSP probably stems from a similar failure of both horns. This discussion refers to PSP and semi-PSP in control matings. Any increase in the treated series must obviously arise from other causes.

It has been amply proven that irradiation of mature sperm has no effect on its powers of fertilisation. In later weeks, however, as shown by Schaefer (1939) the rate of fertilisation is reduced owing to extreme shortage of sperm. PRE is therefore an unreliable measure of dominant lethal mutation rate. Not only are many other factors contributing to it and therefore causing a high error variation, but some of these factors are affected by the treatment itself and thus introduce something much more serious, namely, bias.

Moreover, the existence of PSP presents a special problem. They could be included as PRE, thereby greatly increasing the error variance. But their exclusion is also likely to exclude genuine cases of 100 per cent. dominant lethal incidence, and thus cause underestimation of mutation rate.

ED, on the other hand, seems to have few possible causes other than true dominant lethals. If LD can arise at any time during pregnancy as a result of non-genetic factors, these same factors might be expected to contribute also to ED. But since the total magnitude of LD is small, the corresponding contribution to ED is not likely to be troublesome. Maternal inadequacy in the development of the deciduoma might be expected to produce ED. A small deciduoma

has been seen, incompletely attached to the endometrium, but it was a single instance.

Discussion of ED would be incomplete without mention that similar structures are also seen in pregnancies in which one or both parents are semi-sterile owing to heterozygosity for a translocation. The deciduomata are then supposed to be due to duplication-deficiency zygotes, whose type of chromosome unbalance is somewhat similar to that expected to result from the chromosome breaks in dominant lethal zygotes. No males showed any indication of being semi-sterile. The possibility of a contribution to ED from that source can be excluded.

When *all* implantations are of the ED type, pregnancy terminates on the 12th day, just as for PSP, though there is no confusion with PSP on dissection. The deciduomata undergo a transformation quite different from those in full-term pregnancies. There is no browning of the chorion. The whole structure rounds off and becomes detached from the endometrium as an amorphous pink lump. If the uterus is dissected after the disappearance of the deciduomata it can still be distinguished from PSP by the attachment scars along the mesometrial groove.

There is thus little ambiguity about the recognition or interpretation of ED.

Owing to the unknown and variable frequency of unfertilised ova, it has been considered best to use only post-implantation data for the estimation of mutation rate. From arguments presented elsewhere (Bateman, 1958) the best estimate of mutation rate is taken as the ratio of deciduomata to other implantations, that is, $\frac{\text{ED}}{\text{IMP-ED}}$. This can exceed unity, when it signifies a mean incidence of more than one dominant lethal per sperm.

5. RESULTS

(a) Control matings

For each series of matings of irradiated males, one control male was mated in parallel, to detect any gross variation during the mating period due to environment or to the genetic constitution or age of successive batches of females. No such variation was found. The individual pregnancies are summarised in table 1.

The ED frequency shows a resemblance to a binomial distribution, as would be expected if all pregnancies were from a homogeneous sample. In a few pregnancies there are indications of special factors at work. Thus there was one with 2 ED out of only 8 IMP. This is an abnormally high ratio and appears to be associated with other abnormalities in the same pregnancy, for there were 38 CL, a clear case of spontaneous superovulation, and, probably as a result of the same hormone unbalance, semi-PSP with a low IMP even in the implanted horn. The indications are that the high ED has a maternal

cause and has no connection with mutation. There were two other pregnancies out of the total of 73, which combined low IMP with high ED. Both had normal CL rates (12 and 15) but had only one IMP, which was ED.

TABLE 1

(a) The combinations of PRE and ED found in control pregnancies. Each semi-PSP is indicated by an asterisk

PRE	0	1	2	3	4	5	6	7	8	9	10	11	12 -	15 -	30 -	All (PSP)	Total
ED																	
0	12	10	5	1	1	2*	2*	3*	1		1	1*	1*			6	46
1	7	3		1	1	1				1			1*	1*			16
2	2	4	1												1*		8
3	1		2														3
Total	22	17	8	2	2	3	2	3	1	1	1	1	2	1	1	6	73

(b)

CL per pregnancy . .	10	11	12	13	14	15	16	17	18	19 -	22 -	38
Number of pregnancies .	2	3	9	10	14	14	8	3	3	2	1	1

TABLE 2

Summary of control data. Individual males compared

Male	A	B	C	D	A few pooled		Grand total
Frequencies							
CL . .	263	346	326	162	119		1216
IMP . .	257	291	278	154	112		1092
ED . .	13	14	8	4	6		45
LD . .	9	6	11	7	5		38
PSP . .	2·5	6·5	4	2·5	0		15·5
Matings . .	21	32	28	15	10		106
Percentages						Mean	Weighted mean
PRE/CL .	2·3	15·9	14·7	4·9	5·9	8·7	10·2
ED/IMP-ED) .	5·3	5·1	3·0	2·7	5·7	4·4	4·3
LD/(IMP-ED)	3·7	2·2	4·1	4·7	4·7	3·9	3·6
PSP . .	11·9	20·3	14·3	16·7	0·0	12·6	14·6

On considering PRE, however, we find that even after excluding the above three semi-PSP's, as well as 6 PSP's and 5 additional semi-PSP's, there still remain three pregnancies with many PRE, namely 8, 9 and 10. Clearly, even after excluding grossly abnormal pregnancies, PRE is much more erratic than ED in the control series.

Examination of the control data confirms the conclusion from the earlier theoretical considerations that ED is likely to be superior to PRE for studies on the dominant lethal mutation rate.

TABLE 3

Data after 200 r: all males pooled

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	8-13	Control
Frequencies															
CL . .	352	370	411	254	498	292	214	197	371	220	187	96	213	1284	1216
IMP . .	305	329	315	185	342	130	124	160	331	178	179	89	204	1141	1092
ED . .	67	53	107	34	71	11	13	5	9	10	12	5	9	50	45
LD . .	14	10	10	3	21	10	11	5	8	3	3	9	6	34	38
PSP . .	4	3·5	3	9·5	6·5	30·5	20	4	0·5	1·5	0	0	0	6	15·5
Matings .	30	31	34	31	42	53	39	23	26	12	13	9	16	99	106
Percentages															
PRE/CL .	13·4	11·1	23·4	27·2	31·3	55·5	42·1	18·8	10·8	19·1	4·3	7·3	4·2	11·1	10·2
ED/IMP-ED	28·2	19·2	51·4	22·5	26·2	9·2	11·7	3·2	2·8	6·0	7·2	6·0	4·6	4·6	4·3
LD/IMP-ED	5·9	3·6	4·8	2·0	7·7	8·4	9·9	3·2	2·5	1·8	1·8	10·7	3·1	3·1	3·6
P□P . .	13·3	11·3	8·8	□0·6	15·5	57·5	51·3	17·4	1·9	12·5	□	0	0	□·1	14·6

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In table 2 the control data are presented in the same manner as for the irradiated series, for comparison. PRE varies between males from 2·3 to 15·9 per cent., but both ED and LD are very constant at around 4 per cent. The incidence of PSP (counting semi-PSP as $\frac{1}{2}$) was fairly low, at 14·6 per cent., and the mating rate (per cent. females mated after one week) 94 per cent. PSP and semi-PSP are excluded from all estimations except PSP rate and mating rate.

TABLE 4

ED/(IMP-ED) as a percentage after 200 r: individual values for each male

All matings PSP shown thus : —. No matings : shown blank

Week	1	2	3	4	5	6	7	8	9	10	11	12	13
♂													
1	—	18	37	23	23	—	100	3	4				
2	38	17											
3	50	14	57	—	36	100	22	3	2				
4	31	52	40	10	6	—	0	7					
5	26	8	120	27	35								
6	24	19	52	33	23	9	2	0					
7	65	18	55	—	33	0							
8	16	10	42	34	41	—	33	4	4				
9	14	10	73	8			0	4	0				
10	Kept unmated until week 5					0	—	0					
11						17	—	7					
12						42	0	0					
13						11	50	0					
14	Kept unmated until week 6					7							
15						11							
16						0			2	2	11	8	8
17						6			0	6	4	20	2
18						20			0	7	7	0	6
19						20			8	8	6	5	2
Mean	33·0	18·4	59·5	22·5	24·3	20·3	16·4	3·5	2·5	5·8	7·0	8·3	4·5
Standard error	6·2	4·4	9·6	4·6	4·3	9·1	10·0	0·9	1·0	1·3	1·5	4·3	1·5
Weighted mean (from table 3)	28·2	19·2	51·4	22·5	26·2	9·2	11·7	3·2	2·8	6·0	7·2	6·0	4·6

The mating rate was equally high for all treatments, showing no effect of dose or mating history. No further reference need be made to it.

(*b*) 200 *r*

This dose was chosen as one high enough to give a significant mutation rate, but low enough to allow fertility to continue after week 3, so that mutation rate could be measured over a longer period than studied hitherto. It fulfilled these expectations. The week-by-week analysis of the results is shown in table 3 for all statistics, and in

table 4 for details of IMP and ED alone, arranged so as to show any differences between males. No such differences are evident. The results are shown graphically in figs. 1 and 2.

Fig. 1 shows that, apart from an approximate doubling in week 3, a fairly constant level of mutation is maintained for the first 7 weeks from irradiation. At week 8 the level falls abruptly to the control value and stays there indefinitely.

Fig. 2 shows important differences in the behaviour of PRE and ED. The rise in PRE for week 3, paralleling up to that point the changes in ED, *continues* until week 7. This is due to a reduction in the fertilisation rate for weeks 4 and 5, which becomes very severe in weeks 6 and 7 (see Schaefer, 1939). This low rate of fertilisation corresponds to the complete sterility found during the same period after higher doses such as 500 r.

It will be noticed that the variation in PSP closely follows that in PRE. It is presumably due to the same cause, namely low rate of fertilisation. There seems to be a marked difference here between my own data and Schaefer's (*loc. cit.*), which was obtained at the same dose. In her data, the period of greatest sterility coincided with a *low mating rate*, instead of a high PSP rate. Perhaps in her mice, but not in mine, the urge to mate was conditioned by the amount of sperm in the vas deferens.

Obviously, quite different interpretations of sensitivity to the induction of dominant lethals would be made according to whether one followed implantations alone, or PRE. If one followed both simultaneously (as did Russell *et al.*, 1954) the big increase in PRE over weeks 4 to 7 would swamp the slight drop in ED. As already stated, the author bases his interpretation on ED and IMP alone. On this basis week 3 is hypersensitive to the induction of mutations and the rest of the first seven weeks are about equally sensitive at about half the level of week 3. No induced mutations are recovered after week 7.

A day-by-day breakdown of the data for ED (table 5 and fig. 3) shows that the high value for the third week is not due to an acute phase of short duration, but extends at a constant level throughout the week. On the other hand the slightly raised value for the first week is seen to be due to increased sensitivity for the very first matings in that week. This initial high value of short duration was also found with the data at 125 and 500 r (table 5).

A word is necessary on the LD incidence (see table 3) which showed a slight rise over weeks 5, 6 and 7. This might be related to the low IMP. There are grounds for expecting a connection. It has been found that when there is only one implant, the CL at 12 days are paler than usual on the side bearing the fœtus and white (as in PSP) on the other side. Apparently the stimulus from a single fœtus is not sufficient to promote normal functioning of CL. One might expect uterine function to suffer in consequence. Analysis of the

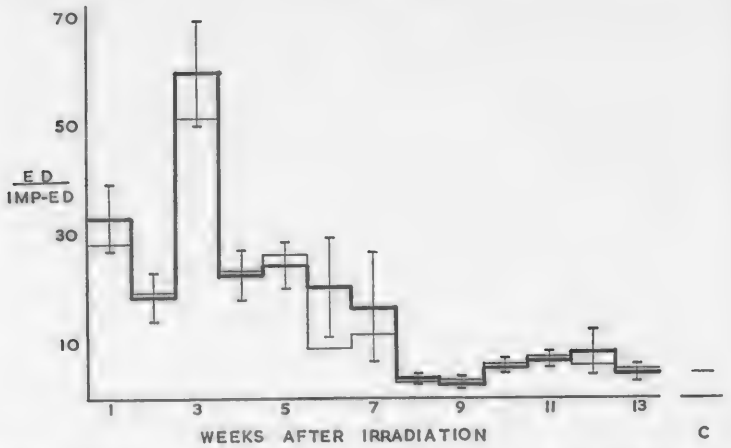

FIG. 1.—Variation of $\frac{ED}{IMP\text{-}ED}$, expressed as a percentage, with successive weeks from the irradiation of the testes of mated males with 200 r. $\frac{ED}{IMP\text{-}ED}$ is the ratio of deciduomata to other implants and is taken as the best measure of dominant lethal mutation rate. Heavy line : weekly mean of values for individual males, with the standard error indicated by the vertical lines. Thin line : weighted mean from pooling data of all males. C : the control value.

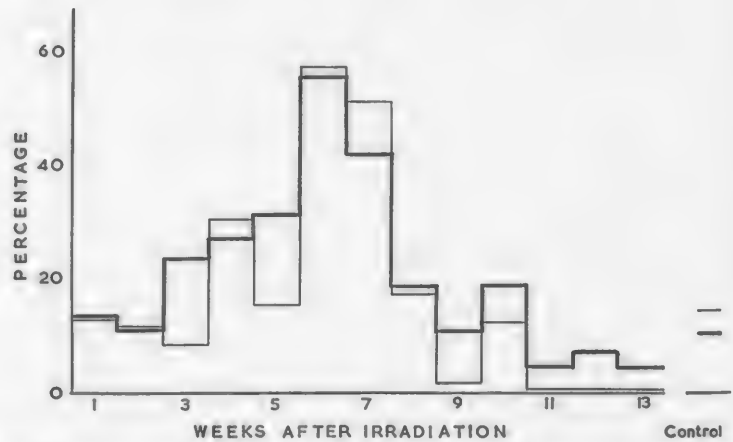

FIG. 2.—Percentage of ova failing to implant in fertile matings (pre-implantation loss = PRE : heavy line) and percentage of sterile matings (pseudopregnancy = PSP : thin line) with successive weeks from irradiation of the testes of mated males with 200 r. Control values for both parameters shown at extreme right.

TABLE 5

Day-by-day variation in ED/(IMP-ED) for the first weeks following irradiation on day 1. Where possible adjacent days with small samples have been pooled to raise the total IMP to 30 or over. Estimates still based on IMP of less than 30 are in parentheses

Day's mating		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Dose 125 r	IMP		32		28	33	24			26				58			46		64		23			
	ED		6		3	2	2			2							9		16		8			
	ED/IMP-ED		23·1		(12·0)	6·5	(9·1)			(8·3)				23·4			24·3		33·3		(53·3)			
200 r	IMP	49	62	54	41	54		77	47	96	34		34		57	27	51	118	36		21		34	44
	ED	14	9	11	7	10		14	4	13	8		8		17	10	19	40	12		3		9	6
	ED/IMP-ED	40·0	17·0	25·6	20·6	22·7		22·2	9·3	15·7	30·8		30·8		42·5	(58·8)	59·4	51·3	50·0		(16·7)		36·0	15·8
500 r	IMP		34	19	59	38		41	19	59			34	35		29			31					
	ED		20	6	20	13		21	11	27			15	31		23			25					
	ED/IMP-ED		142·9	(46·2)	51·3	52·0		105·0	(122·2)	84·4			78·9	775·0		(383·3)			416·7					

pregnancies during this period failed, however, to reveal any correlation between the number of fœtuses per pregnancy and LD.

It may be that during these weeks there is a real increase in the incidence of true dominant lethal *gene* mutations. In that case the sensitivity pattern of the spermatogenic stages is different for chromosome breakage and gene mutation. However, the actual increase in LD is too low in comparison with the control level for it to warrant further consideration.

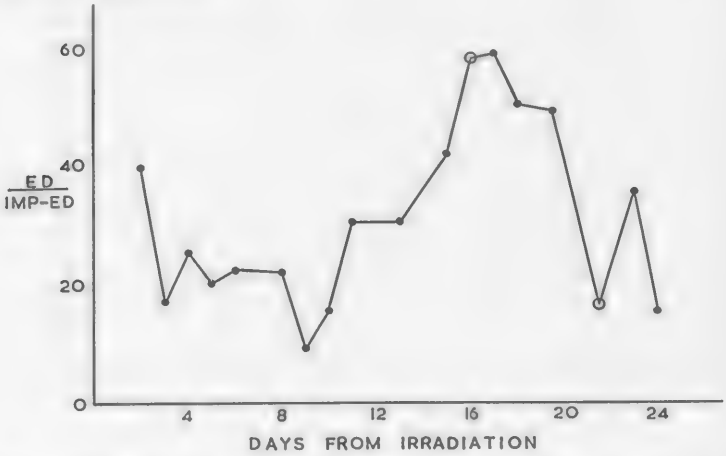

FIG. 3.—Daily values for $\frac{ED}{IMP\text{-}ED}$ following 200 r. All values based on from 30 to 118 implantations except those with the open circle which have 27 and 21 respectively.

(c) 500 *r*

Matings following this dose confirmed the hypersensitivity of the third week after irradiation (table 6). This immediately preceded the prolonged sterility which lasted until the 13th week in a few males kept with females for that length of time.

Reduced but still considerable amounts of motile and apparently healthy sperm can be recovered from the vas deferens in weeks 4 and 5 after 500 r. Sterility during this period is evidently not due to the absence of sperm but to its failure to function properly in fertilisation. Neither is it due to 100 per cent. incidence of dominant lethals, for matings of males given 2000 r were sterile at once, but dissection of the females showed plenty of IMP all of which were ED, which is quite different from the PSP without any IMP found subsequent to the third week after 500 r.

Males given this dose were also used to investigate the possible influence of mating history in determining the mutation rate in sperm samples a given time after treatment. There are several references

to this possibility in the literature, and such an interaction has been found in *Drosophila* (Bateman, 1956*a*).

The results showed no such effect. Males first mated in week 3 showed the same high mutation rate as concurrent matings of males

TABLE 6

Weekly samples after (a) 500 r and (b) 125 r

Week	1	2	3	4	5	6	7
			(*a*) 500 r				
Frequencies							
CL	107*	184*	185	STERILE			
IMP	122	180	95				
ED	53	85	75				
LD	3	8	1				
PSP	4·5	2	8				
Matings	17	22	23				
Percentages							
PRE/CL	33·6*	27·7*	48·6				
ED/(IMP-ED)	76·8	89·5	375·0				
LD/(IMP-ED)	4·3	8·4	5·0				
PSP	26·5	9·1	34·8				
			(*b*) 125 r				
Frequencies							
CL	154	Not	146	Not	159	128	133
IMP	143	sampled	123	sampled	133	110	121
ED	15		32		10	9	11
LD	1		5		8	1	6
PSP	0		0		0·5	2·5	0
Matings	12		12		12	11	10
Percentages							
PRE/CL	7·1	Not	15·8	Not	16·4	14·1	9·0
ED/(IMP-ED)	11·7	sampled	35·2	sampled	8·1	8·9	10·0
LD/(IMP-ED)	0·8		5·5		6·5	1·0	5·5
PSP	0		0		4·2	22·7	0

* CL, and consequently PRE/CL also, for only part of the data.

continuously mated since irradiation. Both series went sterile in week 4. Also, males first mated after week 3 were sterile at once.

It is concluded that there is no storage of sperm in the mouse. This accords with the fact that (unlike *Drosophila*) the male mouse has no organ for the storage of sperm. The "vesiculæ seminales" (better called "albuminiferous glands") merely secrete the material for the vaginal plug. As spermatogenesis is normally a continuous process, it would be sufficient that the newly formed sperm entering the caput epididymis should push out a continuous stream of ripe

sperm from the distal end of the vas deferens, which would be available for any mating which might occur.

This situation greatly simplifies the interpretation of the data. Mating history can be ignored, the sole factor determining the stage at treatment of a given sperm batch being the intervening period of time.

A corollary of this type of sperm flow is that when the pressure from the oncoming sperm is removed, the sperm of previous batches will linger in the vas deferens. It is likely, therefore, that in weeks 6 and 7 after 200 r and 125 r (see below) sperm utilised will have matured the previous week.

(*d*) 125 *r*

Some doubt in the interpretation of the 200 r data arises from the high error variation in weeks 6 and 7, owing to the high sterility and therefore small size of sample. A lower dose (125 r) was therefore chosen, sufficient to give a detectable mutation rate, but showing no sterility in weeks 6 and 7. As will be seen from table 6, after the expected peak in week 3, a constant mutation rate, approximately equal to that in week 1, extended over weeks 5, 6 and 7.

6. SPERM SAMPLE EQUATED TO STAGE IRRADIATED

The variation in mutation rate with time of sampling is quite clear. Its interpretation in terms of the stages of spermatogenesis irradiated is more problematical. Fortunately there has recently been a number of investigations on the timing of the various stages of spermatogenesis (Leblond and Clermont, 1952 ; Sirlin and Edwards, 1955, and Oakberg, 1957). These have been synthesised into a single scheme by Sirlin and Edwards (1957).

Oakberg (1955) has shown that the later cell generations of spermatogonia are much the most sensitive to killing by irradiation : only 2 per cent. survive 100 r. It is reasonable to suppose, therefore, that the high sterility of weeks 6 and 7 after 200 r is due to the annihilation of spermatogonia. The fortnight of weeks 4 and 5 would then be sampling the products of irradiated spermatocytes, which are known from histological observation to persist for a fortnight after irradiation (*i.e.* spermatocytes have a life of 14 days). These assumptions fit the time sequence shown in fig. 4 and derived from Sirlin and Edwards (1957).

This timetable is based on the following data :—

1. Oakberg's (1957) estimate of 34·5 days for the complete spermatogenic cycle from stem cell to the liberation of morphologically mature sperm from the seminiferous tubules. This was partitioned between the stages as follows : spermatogonial mitoses, 6 days ; spermatocytes, 14 days ; spermatids, 9 days ; and sperm 5·5 days. The dividing line between spermatids and sperm is more arbitrary than the others

as metamorphosis is continuous, without a nuclear division to mark the transition.

2. Sirlin and Edwards' (1955) observation of 8 days from the last DNA synthesis to the appearance of labelled DNA in spermatids, and a further 22 days to its appearance in the ejaculate.*

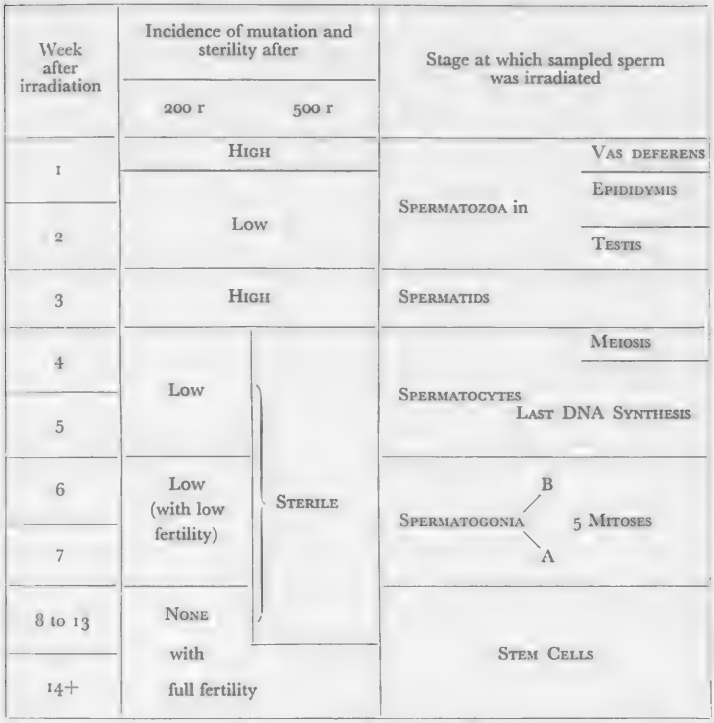

Fig. 4.—An attempt to equate the sequence of matings following irradiation, in respect of dominant lethal mutations and fertility, with the known spermatogenic timetable.

3. By combining these independent sets of data we can calculate that the last DNA synthesis occurs in 6-day-old spermatocytes, and that the time taken for the sperm to travel from the testis to the ejaculate is 7·5 days $(22-(9+5{\cdot}5))$.

It is remarkable that Schaefer (1939) had postulated, largely intuitively, the *same* time scale for the interpretation of her 200 r data.

One concludes from fig. 4 that while, after 200 r or 125 r, spermatocytes are capable of producing fully functional sperm, they do not do so after 500 r.

* There is frequent confusion over terminology in the literature, early or "resting" spermatocytes being referred to as spermatogonia type B ("crusty"). Thus these authors refer the last DNA synthesis to the spermatogonia, though it must occur in meiotic prophase, just prior to pachytene, when the spermatocyte becomes "tetraploid".

The statement of Auerbach (1957) that 500 r kills spermatocytes is wrong. They persist, in diminishing numbers, as those passing out via meiosis are not replenished from the non-existent spermatogonia type B, for a fortnight, by which time they have all produced spermatids. 500 r does not even kill all the products of division of these spermatocytes. Whilst many obviously abnormal spermatids are produced by such spermatocytes, a large number appear to be normal and metamorphose into normal-looking sperm, which can be found, alive and motile though in distinctly subnormal frequency, in the vas deferens of a male who has been mated continuously for five weeks after irradiation, though he is now quite sterile.

It would appear then that sperm from spermatocytes which have received 500 r is incapacitated in some subtle way.

200 r does not appear to have this effect, though irradiated spermatocytes still produce many abnormal spermatids and malformed and necrotic sperm. The reduced rate of fertilisation in weeks 4 and 5 may be due to the incapacity of some of the sperm induced at the lower doses by the same mechanism as operates after 500 r. It is likely that in weeks 6 and 7 after the lower doses the sperm utilised is still that which was irradiated as spermatocytes (see earlier discussion).

This leads to the conclusion that irradiated meiosis is sampled as sperm at the end of the third week, though it is too short-lived a stage to be individually identifiable. The week-long hypersensitive period is, therefore, due to irradiation of spermatids at all stages from their origin to a stage where they become morphologically similar to sperm (Leblond and Clermont's "maturation stage"). This is about midway between meiosis and the liberation of the sperm from the tubules. For the rest of the time spent in the testis, and during the passage down the epididymis there is no change in sensitivity, though there is a final peak in sensitivity just before ejaculation which can probably be referred to sperm in the vas deferens.

The data here presented confirm the hypersensitivity found in the third week from irradiation by Kaplan and Lyon (1953), Russell *et al.* (1954) and Auerbach and Slizynski (1956). It should not be confused, however, with the increase in mutation rate found in the *second* week by Snell (1933), Brenneke (1937) and Hertwig (1938). A feature common to these workers was the high dosage level (800 r) which produced sterility in the third week, a sterility in all probability due to 100 per cent. incidence of dominant lethals and therefore quite distinct from that in the fourth week after 500 r.

7. PRODUCTS OF IRRADIATED SPERMATOCYTES

I had earlier (1956*b*, *c*) fallen into the error of assuming that "dominant lethals" (*i.e.* chromosome breaks) induced in spermatocytes, which would be expected to result in aneuploid spermatids, would make the sperm inviable and so unable to be recovered from

fertilised eggs. This assumption is, of course, in direct contradiction to the interpretation of semi-sterility as due to the death of duplication-deficiency zygotes, which could only be produced from viable duplication-deficiency gametes. If the unbalanced sperm were non-functional (this theoretical possibility was pointed out by Koller in 1944, especially when the interchanged segments are large), males heterozygous for the translocation would cease to be semi-sterile, for although half the sperm would be inviable, there would still be the other half to fertilise all the eggs, and all the zygotes would then be viable.

Many effects of radiation on spermatocytes, however, such as bridges at first or second division, causing restitution, are equivalent to dominant lethals in mode of origin and will produce inviable sperm, which can be seen in irradiated testes. Consequently, sperm sampled in weeks 4 to 7 after 125 r and 200 r is not a complete sample of irradiated spermatocytes. It is likely to show less than the primary incidence of dominant lethals. The sensitivity of spermatocytes to mutagenesis is likely to be a minimal one when measured by dominant lethals.

There is evidence (Sparrow, 1951) that the pollen mother cells of plants are much more sensitive to chromosome breakage than other stages. If this is a property of meiotic chromosomes as such, it is likely to apply equally in plants and animals. A true measure of the mutagenic sensitivity of spermatocytes will only be found using mutations which are not, like dominant lethals, subject to germinal selection.

8. THE STERILE PERIOD

Although the later spermatogonial generations are destroyed by 200 r full fertility is restored by week 8 and maintained at least until week 13. This raises the problem of how to explain the prolonged sterile period after doses such as 500 r. If there are any stem cells left alive at all, one would expect, from the spermatogenic timetable of Oakberg, that they would yield viable sperm after 40 days (6 weeks), whereas in fact one has to wait yet another 6 weeks for the return of fertility. Why is this?

Oakberg (1955) found that after 600 r the surviving stem cells did not begin to repopulate the seminal epithelium until more than a week had elapsed, and it was three weeks before spermatogonia type B reappeared in quantity. Evidently the spermatogenic cycle does not start up at normal speed immediately after irradiation. Even after the establishment of the spermatogonial population spermatogenesis must be retarded, to account for the further 10-week delay to the restoration of fertility.

Thus, whilst the rate of spermatogenesis of the post-spermatogonial stages is little affected by radiation, the rate of subsequent recovery of the spermatogenic cycle is greatly retarded by high doses (500 r and above).

This retardation is all the more striking if one considers that in males of the same F_1 strain, full fertility is attained at an age of 6 weeks, only 9 weeks from conception, during which time not only spermatogenesis is completed but also the entire ontogeny of the genitalia.

An extreme form of the same phenomenon may account for reports that even longer periods, measured in years, are required for the restoration of fertility to men given temporarily sterilising doses of X-rays (Glucksmann, 1947).

9. COMPARISON WITH *DROSOPHILA*

According to the above interpretation of the mouse data and my own *Drosophila* results (1956*a*, 1957*b*), both organisms show a hypersensitive stage immediately following meiosis and extending to the transformation of the spermatids into sperm. There is a quantitative difference, however, for the ratio of sensitivity of spermatids to sperm is 10 in *Drosophila* but only 2 in the mouse. This may be related to the much greater attenuation of the sperm head in *Drosophila*, the ratio of sensitivity being determined by the degree of change involved in the transformation.

It is not surprising that the products of irradiated spermatocytes have never been recognised in *Drosophila*, for in the adult they are a very short-lived stage. No comparison can be made, therefore, between the sensitivities of this stage for the two organisms.

There does seem to be a difference in the sensitivity of spermatogonia to the direct lethal effect of X-rays, for there is no evidence in *Drosophila* of the destruction of this stage by doses less than those required for 100 per cent. incidence of dominant lethals in mature sperm. After 3000 r fertility is normal from the ninth day (Bateman, 1956*a*). Whether there is a corresponding difference in sensitivity to induction of mutations is an open question.

10. SUMMARY

1. Males with a testis dose of 200 r were mated to four virgin females per week for up to 13 weeks from irradiation, and the females dissected at 12 days.

(*a*) Pre-implantation losses and pseudopregnancies rise from week 2 to weeks 6 and 7, and then fall abruptly to the control level for the rest of the period studied. The cause is partly the shortage of sperm for weeks 4-7 which is particularly acute for weeks 6 and 7.

(*b*) Deciduomata, on the other hand, show a peak incidence extending over the whole of week 3, with about half this level both before and after up to week 8, when the incidence falls to the control level. There is also a peak at the beginning of week 1.

(*c*) Fœtal deaths are at the control level throughout.

2. The 200 r data are confirmed by doses at 125, 500 and 2000 r.

3. The unmated male mouse does not store sperm. Consequently, mating history has no effect on the mutation rate recovered a given interval from irradiation.

4. The dominant lethal mutation rate has been estimated as the ratio of deciduomata to other implants.

5. Estimated mutation rates were :

(*a*) for controls and for more than 7 weeks from irradiation ; 0·04.

(*b*) for the first seven weeks from irradiation (except week 3) 0·12 for 125 r ; 0·28 for 200 r ; and 0·77 for 500 r.

(*c*) for week 3 : 0·35 for 125 r ; 0·51 for 200 r ; and 3·75 for 500 r.

6. The various sampling periods after 200 r and 125 r have been identified with the products of the following irradiated stages :

Period	Stage irradiated	Sensitivity	
		Mutagenic	Other
Beginning of week 1	Sperm in vas deferens	High	Nil
Rest of week 1 and week 2	Sperm, first in epididymis, later in testis	Standard	Nil
Week 3 .	Spermatids	Doubled	Nil
Weeks 4 and 5	Spermatocytes	Minimal estimate equal to standard	No immediate effect but resulting sperm partially incapacitated
Weeks 6 and 7	Spermatogonia	No estimate possible	Completely destroyed. The sample during this period consists of residuum from spermatocytes
Weeks 8 and later	Stem cells	No dominant lethals recoverable	Fairly resistant

7. The prolonged sterile period after 500 r is due to the following successive factors : complete impotence of sperm in weeks 4 and 5 ; destruction of spermatogonia in weeks 6 and 7 ; retardation of the recovery of normal spermatogenic cycle for a further 6 weeks or so.

8. In both *Drosophila* and mouse the hypersensitive stage is the spermatid, though the relative sensitivity is 5 times greater in *Drosophila*.

Acknowledgment.—Thanks are due to Miss Veronica Baccas for technical assistance.

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UNILATERAL INTERSPECIFIC INCOMPATIBILITY IN FLOWERING PLANTS

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1. INTRODUCTION

THE five well-established systems of sexual incompatibility in the flowering plants differ greatly in their genetical and physiological control. Each system during its evolution has accumulated its own special secondary differences in the genetic and floral structure of the species, culminating in a whole complex of integrated genetical and physiological characters. Because these complexes are very different in the different incompatibility systems, it is improbable that a switch in evolution from one fully developed system to another could occur.

A similar conclusion is inescapable from a study of the way the systems are distributed in taxonomic groups. Whole families have only one system as shown by heterostyly in the Primulaceæ and Plumbaginaceæ, gametophytic homomorphy in the Solanaceæ, Scrophulariaceæ and Rosaceæ, sporophytic homomorphy in the Compositæ and Cruciferæ. Even closely related families, the Solanaceæ and Scrophulariaceæ, have the same basic system.

In many species with gametophytic incompatibility, even when they come from different families, the genetics of the system is identical down to such details as the kinds of mutation of the controlling genes that occur. The physiology of incompatibility is also basically similar in such families: *Œnothera organensis* in the Onagraceæ and *Petunia hybrida* in the Solanaceæ, and probably others, have an antigen-antibody type of reaction between pollen and style (*cf.* Lewis, 1952; Linskens, 1953).

In closely related species the physiological control is actually identical in all its details. This is shown by hybrids of *Œnothera* species (Crowe, 1955) in which incompatibility works just as efficiently as in the species themselves. But widely different families, although they have the same basic antigen-antibody reaction, may differ in detail such as the effect of temperature. In *Œnothera* species the incompatibility reaction is accelerated by increased temperature, while in *Petunia* species it is not.

These comparisons between self-incompatible species are based on (1) a detailed analysis within the species, which is enough to characterise the basic genetic and physiological type and (2) an analysis of interspecific hybrids which shows whether or not the incompatibility

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systems in different species are identical in their genetic and physiological control.

The more distant relationships between species, genera and families, however, are outside the scope of such tests, which depend on the production of fertile hybrids, but the reaction of pollen and style provides a method of extending the analysis to groups that do not hybridise.

So far we have discussed only self-incompatible species. The majority of families and genera contain both self-incompatible and self-compatible species. Style-pollen relationships between a self-incompatible and a self-compatible species have been reported in the literature. In some examples a remarkable phenomenon was found. The pollen of the self-compatible (SC) species was inhibited in the styles of the self-incompatible (SI) species, while no inhibition occurred in the reciprocal cross. This unilateral incompatibility was totally unexpected on the current theories, and was therefore of great interest. But there were just as many exceptions in which the pollen of the SC species was not inhibited ; and therefore, interesting as the phenomenon was, it appeared not to be general and consequently not basic.

The data presented in this article show in fact that unilateral incompatibility of the type, SI ♀×SC ♂, is general. The clue to its meaning and to the significance of the exceptions, which prove the rule, has come from mutation studies of the S locus.

The unilateral phenomenon is undoubtedly basic to the incompatibility system : it should prove to be a powerful tool in analysing distant phylogenetic relationships because it can be demonstrated without the production and fertility of a hybrid. It should be a means of estimating the age of incompatibility systems, and it is a fact to be considered in any theory of the genetics and physiology of the systems.

2. MATERIALS AND METHODS

The species were grown from seed obtained from various Botanical Gardens and seed firms as follows :

Cruciferæ	12 species, details in Bateman, 1955.	
Onagraceæ	10 *Œnothera* species, details in Crowe, 1955.	
	Epilobium angustifolium Linn.	Thompson and Morgan, Ipswich
	Epilobium hirsutum Linn.	Thompson and Morgan, Ipswich
	Epilobium lanceolatum Seb and Maur.	Thompson and Morgan, Ipswich
	Godetia amœna G. Don.	Chelsea Physic Garden
	Œnothera biennis Linn.	Chelsea Physic Garden
	Œnothera erythrosepala	Instituto Botanico, Coimbra, Portugal
	Œnothera lamarckiana	Thompson and Morgan, Ipswich
	Œnothera rosea	Portugal
Solanaceæ	*Capsicum annum* Linn.	Thompson and Morgan, Ipswich
	Capsicum frutescens Linn.	Thompson and Morgan, Ipswich
	Lycium chinense Mill.	Chelsea Physic Garden
	Lycopersicon esculentum Mill.	John Innes Hort. Instn.

Lycopersicon var. Best of All	John Innes Hort. Instn.
Lycopersicon hirsutum	Peru
Lycopersicon peruvianum	Peru
Lycopersicon pimpinellifolium	John Innes Hort. Instn.
Solanum capsicastrum Link.	John Innes Hort. Instn.
Solanum jasminoides Paxt.	John Innes Hort. Instn.
Solanum melongena Linn.	Thompson and Morgan, Ipswich
Solanum pseudocapsicum Linn.	Thompson and Morgan, Ipswich
Solanum rybinii Juz et Būk	John Innes Hort. Instn.

Several individuals of each species, at least four, were tested for self-incompatibility by seed setting and pollen-tube growth. Acid fuscin- light green mixture was the stain used in the Cruciferæ, Solanaceæ and Scrophulariaceæ; the iodine-alcohol method was used in the Onagraceæ.

For measurement of pollen-tube growth flowers were pollinated, cut from the plant, placed in water and incubated at 25° C. for most of the tests; when the effect of temperature was the object of the test the details are given in the text. Pollen-tube measurements were made on the longest pollen tube in the style.

3. INTERSPECIFIC RELATIONSHIPS

(i) *Gametophytic homomorphic system with one locus*

Solanaceæ. The genera and species in the Solanaceæ were selected to include a range of SI and SC species. In pollinations within species, pollen tubes of SC species and cross-compatible tubes of SI species grew to the base of the style, a distance of 6-8 mm. in 24 hours, and continued to grow into the ovary. The differences in growth rate of the pollen tubes between the species was not large and was correlated with the size of the pollen grain, which in turn was correlated with the length of the style. But these differences between the species are of no significance in the present study. Selfed pollen tubes of SI species grew 4·2 mm. in 24 hours in *Lycopersicon peruvianum* and *Lycopersicon hirsutum* and 3·0 mm. in *Solanum jasminoides*: no further growth occurred. Thus the difference between incompatible and compatible growth in these species was large and consistent. Selfed pollen tubes of *Solanum rybinii*, however, grew almost as fast as compatible tubes, attaining a length of 6-8 mm. in 24 hours.

The growth of the pollen tubes in interspecific and intergeneric crosses, given in fig. 1, are classified as +, when the tubes are 6-8 mm. long and into the ovary, or —, when they are less than 3 mm. long.

Fig. 1 shows that seven species of the three genera, *Capsicum*, *Lycopersicon* and *Solanum* are self-compatible (SC). The pollen of these species, when intercrossed on to any of the other SC species, is not inhibited. But when this SC pollen is placed on styles of the four SI species it is strongly inhibited. In contrast, pollen from SI species is not inhibited in styles of other species whether they are SI or SC. *Lycium chinense*, although it is SC in our material, has been included in the SI group for reasons that will be apparent later. *Solanum rybinii* is not included in the figure because it behaves in a unique way which we cannot explain. It is SI and its style behaves accordingly by inhibiting pollen of SC species. But its pollen on

the contrary is inhibited in SI styles of other genera. This abnormal pollen behaviour may be associated with the fact that the self-pollen tube growth in *S. rybinii* is different from that in the other SI species of the Solanaceæ, for it continues to grow slowly to the base of the style.

Scrophulariaceæ. Eight species of *Antirrhinum*, including SI and SC species, have been investigated by Harrison and Darby (1955). As in the Solanaceæ, the two SC species *A. siculum* and *A. orontium* have

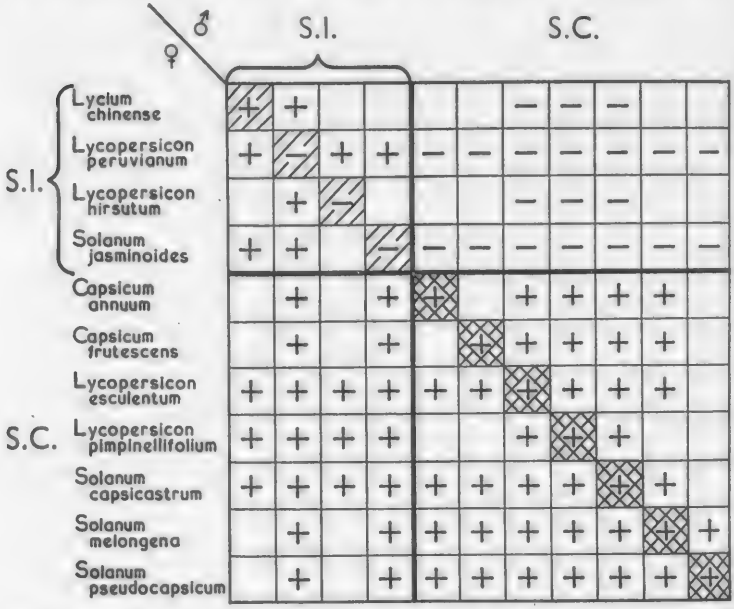

FIG. 1.—Results of intercrossing self-incompatible (SI) species with self-compatible (SC) species in the Solanaceæ: —pollen tubes are inhibited in top 3 mm. of the style, + pollen grows into the ovary.

their pollen tubes inhibited in the styles of the SI species. In all other combinations SC × SC, SC × SI and SI × SI the pollen tubes are not inhibited.

Solanaceæ × *Scrophulariaceæ*. In view of the inhibition of SC pollen in SI styles in both the Solanaceæ and the Scrophulariaceæ, we made tests by cross-pollinating between the two families. If a similar inhibition existed between the families a common cause of the phenomenon could be established.

Pollen of Solanaceous species when applied to the *Antirrhinum* species did not germinate in most cases, and in none did the tubes penetrate the stigma. The results are given in table 1.

It is not surprising that the physico-chemical nature of the stigmas in the two families is apparently different. Such gross differences are probably independent of the differences between SI and SC styles.

However, *Antirrhinum* pollen germinated and grew normally in the styles of the Solanaceæ. Pollen tubes were frequently present in

TABLE 1

Results of Intercrossing the Scrophulariaceæ *with the* Solanaceæ

Antirrhinum 4 species (SI and SC) × Solanaceæ 4 species (SI and SC)
Pollen does not germinate

Antirrhinum meonanthum × *Capsicum annuum*

Antirrhinum meonanthum × *Capsicum frutescens*
Tubes 2 × diameter of pollen

Antirrhinum meonanthum × *Lycopersicon esculentum*
Tubes 2 × diameter of pollen—burst

the ovary. The results of these inter-family tests are given in fig. 2. Despite some complications, the main features are unambiguous.

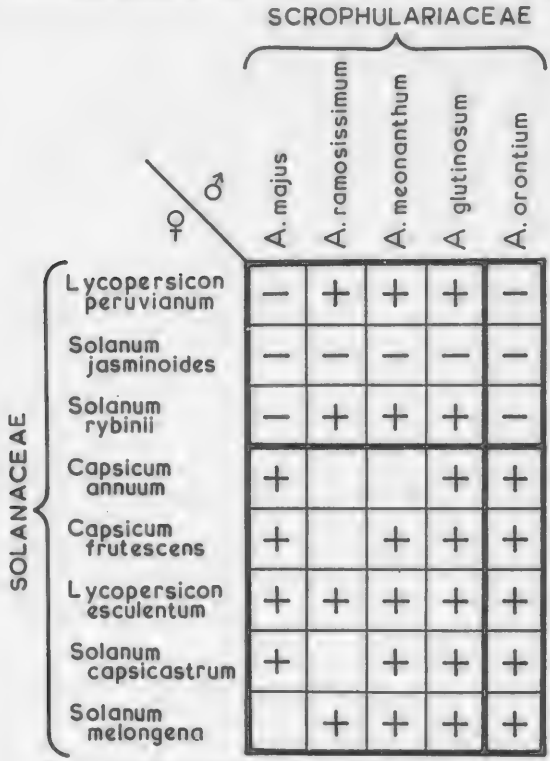

FIG. 2.—Results of intercrossing pollen of *Antirrhinum* species on to the Solanaceæ.

The pollen tubes of the three SI species, *A. ramosissimum*, *A. meonanthum* and *A. glutinosum* were not inhibited in SI or SC species of the Solanaceæ. Pollen of the SC *A. orontium*, however, was inhibited in the SI species but not in the SC species of the Solanaceæ. Thus

the inter-family crosses follow the pattern established by crosses within the family. One complication was that pollen tubes of *Antirrhinum* species were unable to grow in the style of *Solanum jasminoides*. This inability to grow is visibly different from the (SI ×SC) inhibition since the tubes were extremely short, swollen and sometimes burst.

Exceptional behaviour was also shown by *A. majus* pollen. On the styles of other species of *Antirrhinum* it behaved like an SI species by growing uninhibited in styles of both SI and SC species. But on SI styles of the Solanaceæ it was inhibited. Because *A. majus* has only recently become self-compatible by mutation and selection this different reaction on the styles of its own genera and on the Solanaceæ may be significant. *A. majus* occupies an intermediate position between SI and SC species, and it shows a similarly intermediate state in regard to its reaction with other genera.

Onagraceæ. The species tested in the Onagraceæ can be conveniently grouped as follows :

8 SI species of Œnothera without established chromosome rings
6 Sc * species of Œnothera which have close relatives that are SI
3 Sc species of Œnothera, *O. biennis*, *O. lamarckiana*, *O. erythrosepela* which are self-fertilising and have established ring-forming chromosomes
3 species of Epilobium which is an SC genus
1 species of Godetia which is an SC genus

The results, given in fig. 3, show that in no combination was the pollen of either an SC or an SI species inhibited. The exceptions with *Epilobium hirsutum* and *Godetia amœna* are not relevant. These styles would not allow pollen of any foreign genera to germinate and therefore the negative results indicate gross physiological differences rather than incompatibility.

The actual growth of the pollen of different species varied greatly, but when a comparison was made of the growth of the pollen on its own and a foreign style, it was evident that there was no inhibition. Certain styles such as those of *Œnothera pallida* appeared to be less favourable for the growth of Epilobium pollen than those of other Œnothera species, but again little significance can be attached to this.

(ii) *Sporophytic homomorphic system*

The Cruciferæ have a system of incompatibility controlled by a single gene with many alleles as in the system found in the previous families. But the pollen reaction is under sporophytic control and not under gametophytic control. It is therefore of interest to determine whether the (SI ×SC) inhibition, found in the gametophytic system, operates in other systems.

Bateman (1955) in his survey of the family for self-incompatibility found 80 SI and 102 SC and Sc species. Most of the genera have

* See appendix, p. 254.

both SI and Sc species; only one, *Iberis*, was wholly SI and four *Lepidium*, *Æthionema*, *Draba* and *Eriophila* were wholly SC as far as the species tests showed.

The interspecific and intergeneric crosses are shown in fig. 4. Six SI species and five Sc species taken from mixed SI, Sc genera, and one species each from the SC genera *Æthionema* and *Draba* have been tested.

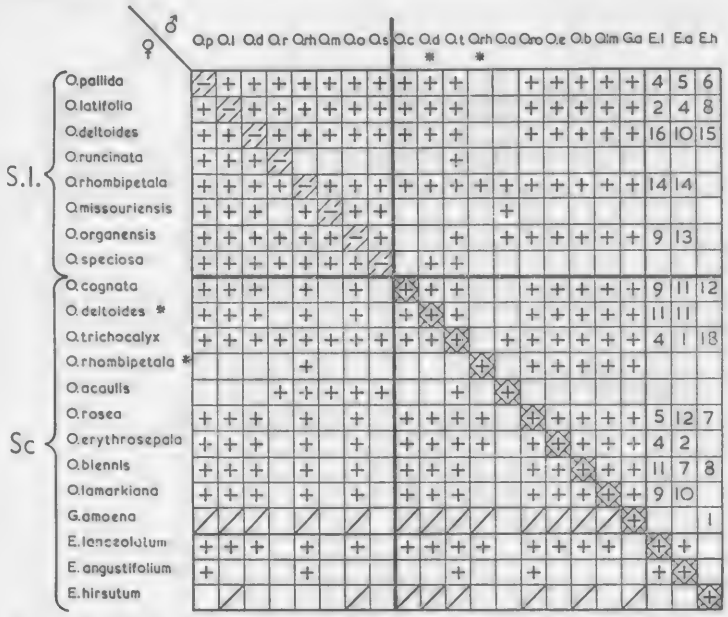

FIG. 3.—Results of intercrossing in the Onagraceæ; *Sc forms of an SI species. The figures in the last three columns give the lengths in mm. to which pollen tubes grow in 24 hours.

It is clear from the figure that pollen was inhibited only when *Æthionema* pollen was placed on the style of other species. The styles of the other SC species *Draba verna* did not inhibit the pollen of *Æthionema*.

This result is therefore similar to the results in the preceding families with gametophytic incompatibility, suggesting that the (SI × SC) inhibition is also a feature of sporophytic systems. The explanation for the non-conformity of the pollen of four Sc species in SI styles will be discussed later, along with the exceptional *A. majus* and *Lycium*.

In passing, however, we can note that Riley (1932) also recorded results in *Capsella* which are, at first sight, a violation of the (SI × SC) rule. In crossing *C. grandiflora* SI reciprocally with *C. rubella* (Sc) and with *C. virginirri* (Sc) all crosses were compatible.

Karpechenko (1928) and many others obtained hybrids between *Raphanus sativus* (SI) and *Brassica campestris* (SI) only when the *Brassica* was used as pollen. The pollen tubes of the *Raphanus* were reported to be inhibited in the *Brassica* styles. Furthermore the allotetraploid of this hybrid, *Raphanobrassica*, was unexpectedly found to be self-compatible, and showed some unusual behaviour when back-crossed with the parents.

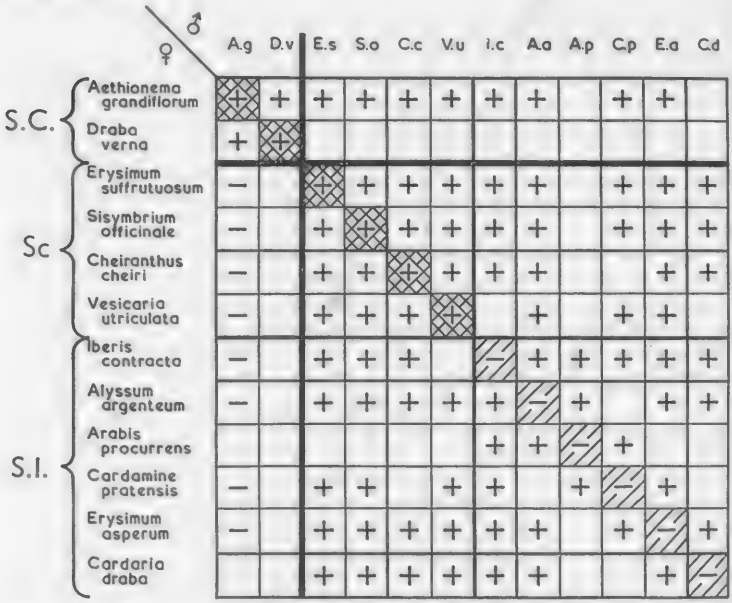

FIG. 4.—Results of intercrosses between species and genera in the Cruciferæ; SC self-compatible species from genera that are wholly self-compatible, Sc self-compatible species from genera that contain SI species.

(iii) *Heteromorphic system*

The Linaceæ and Primulaceæ contain SI heterostyled and SC homostyled species. Tammes (1928) found that the SC homostyled species, *Linum usitatissimum*, was compatible when used as a female with pollen of five different SI heterostyled species. The reciprocal cross using the SC species as pollen was incompatible. A similar result was obtained with the SC homostyled, *Linum monogynum* (Vargas Eyre and Smith, 1916).

Khanna and Singh (1956), examined pollen tube growth in crosses between *Linum* species. They used two heterostyled species *L. grandiflorum* and *L. africanum* and crossed them with the homostyled *L. usitatissimum*. Pollen tubes were normal and uninhibited when the homostyled species was used as the female, but when used as the pollen

parent the tubes were swollen and sometimes burst and never penetrated beyond the middle of the style. Khanna and Singh explain the inhibition on the difference in chromosome number of the species. *L. grandiflorum* has $n = 8$, *L. usitatissimum* with $n = 16$, *L. africanum*, $n = 15$. A more probable explanation based on (SI ×SC) inhibition which is concerned with pollen-tube growth appears to be more satisfactory.

In the Primulaceæ, Ernst (1953) distinguishes between primary and secondary homostyly. The secondary homostyles are recent mutants in an otherwise heterostyled species. Several of these mutants have occurred in experiments. These homostyles when crossed with heterostyled individuals obey the incompatibility rules which are based upon the known reactions of pollen and styles of the different morphological types. The homostyled species, however, do not conform with the rules. For example, crosses between the heterostyled SI *P. pulverulenta* and the homostyled SC species *P. cockburniana* and *P. chungensis* are compatible in all combinations. The evidence in the heterostyled species consists therefore of two examples of (SI ×SC) inhibition in *Linum* and no inhibition in *Primula*. Again the data support and do not conflict with the (SI ×SC) rule for the heterostyled system.

(iv) *Gametophytic homomorphic system with two loci*

Self-incompatibility is common in the Gramineæ, and in the only two species that have been examined, *Secale cereale* and *Phalaris cœrulescens*, the system is controlled by two loci S and Z both with multiple alleles and gametophytic control of the pollen (Lundqvist, 1954; Hayman, 1956). Many interspecific and intergeneric hybridisations have been made in the Gramineæ, and there are two examples of a seed failure in the (SI ×SC) cross where the (SC ×SI) is fertile. These are *Secale cereale* ×*Hordeum jubatum* (Quincke, 1940), and *Phleum alpinum* ×*P. nodosum* (Nordenskiold, 1937). But in neither of these cases is it known with certainty that the sterility of the (SI ×SC) cross is due to pollen-tube inhibition.

(v) F_1 *hybrids*

Another important aspect of the interspecific relationships is the behaviour of F_1 hybrids. In the gametophytic system, a cross SC × SI such as *Lycopersicon esculentum* ×*L. peruvianum* (McGuire and Rick, 1954), assuming that the two species have an homologous incompatibility (S) locus, the F_1 will contain one S allele from each parent, and can be represented as S1Sc. These alleles will segregate in the pollen of the hybrids giving S1 and Sc pollen grains. On self-pollination S1 pollen tubes will be inhibited because S1 is also present in the style. This inhibition is expected on the rules governing the behaviour in homomorphic incompatibility systems. Sc pollen tubes will also be inhibited by the presence of S1 in the style, but we

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attribute this reaction to the (SI ×SC) inhibition. The exceptional Sc *Antirrhinum majus* and the Sc *Œnothera* species, which have pollen that is not inhibited in an SI style, also differ in their F_1 hybrids with SI species. They are self-compatible (Shermann, 1939 ; Crowe, 1955). Predictable as this behaviour of the F_1 is in the gametophytic system, this is not so in sporophytic systems owing to the complication of dominance in the pollen. Even within a species, which is an uncomplicated relationship, an **Sc** allele can be either dominant or recessive according to the particular SI allele accompanying it (Bateman, 1954).

However, in crosses between species an **Sc** allele which shows (SI ×SC) inhibition should produce a self-incompatible F_1 hybrid whether the **Sc** allele is dominant or recessive. If **Sc** is dominant all the pollen will be phenotypically SC, and this will not function on an **S1Sc** style. If, on the other hand, **Sc** is recessive all the pollen will be phenotypically **S1** and this will be opposed by the same **S1** allele in the style. Only if the **Sc** allele has reversed dominance in pollen and style will the F_1 be self-compatible.

Capsella species, in which the **Sc** allele does not have the (SI ×SC) inhibition, produce F_1 hybrids that are self-compatible (Riley, 1932), as expected if **Sc** is dominant in the pollen.

The Compositæ also has the sporophytic system. Our limited tests in the family include *Cosmos bipinnatus*, *Cosmos diversifolium*, *Layia platyglossa*, *L. pentachadii* and *Senecio vulgaris*, but pollen in all crosses did not germinate. An example in the literature is found in the genus *Crepis*. *Crepis fœtida* has an incompatibility system with sporophytic control and many alleles of the S gene (Hughes and Babcock, 1950). This species exists in SI and Sc varieties (Babcock and Cave, 1938). The cross between these two varieties is compatible in both directions, the F_1 is SI and the F_2 segregates SI and Sc plants. This is in agreement with the Sc variety being a recent mutant of the SI variety, with the Sc allele dominant in the pollen. Two SC species *C. thompsonii* and *C. eritreensis*, however, will cross with the SI, *C. fœtida*, only in the direction SC ×SI. The reciprocal cross presumably shows (SI ×SC) inhibition. The F_1, with two exceptions, is SI. This result agrees well with the expectations. But before it can be said with any degree of certainty that the (SI ×SC) rule applies to the Compositæ, some critical tests of it must be made in this family.

4. PHYSIOLOGY OF INTERSPECIFIC INCOMPATIBILITY

(i) *Strength of the (SI×SC) inhibition*

In the gametophytic system, incompatible pollen tubes stop growing at varying times after pollination and at varying distances down the style, according to the species and conditions. But the time and place of inhibition is fairly narrowly defined and characteristic for each species.

The self-pollen tubes of *Lycopersicon peruvianum* at 26° C. reached a mean length of 4·2 mm. after 24 hours. The mean length of pollen tubes of six SC species of the Solanaceæ growing in *L. peruvianum* was only 1·7 mm. The maximum was 3·0 for *Solanum capsicastrum* and 0·1 for *Capsicum frutescens*.

McGuire and Rick (1954) found the same difference between SI ×SC inhibition of *L. peruvianum* ×*L. esculentum* and self-inhibition of *L. peruvianum*. Bateman (1943) found this difference in *Petunia* species. All these examples are in the Solanaceæ, but in the Scrophulariaceæ a similar difference exists. *Antirrhinum meonanthum*, SI, when selfed at 26° C. produces pollen tubes 8·5 mm. long in 26 hours. The SI ×SC cross *A. meonanthum* ×*A. orontium* produces tubes only 1 mm. long. *A. glutinosum*, however, shows equal and strong inhibition in selfing and in crossing with *A. orontium* pollen. The pollen tubes grow only 1·5 mm. at 15° C.

We can conclude that for the gametophytic system, at least in the Solanaceæ and Scrophulariaceæ, the SI ×SC inhibition is stronger or earlier in action than the inhibition of selfing.

A similar comparison could not be made for the Onagraceæ because there was no example of (SI ×SC) inhibition, or in the sporophytic system of the Cruciferæ because the self and (SI ×SC) inhibitions are so immediate as to prevent germination.

(ii) *Temperature and the inhibition reaction*

In all SI species tested in the Onagraceæ the strength of the inhibition on selfing is increased with increasing temperature; $Q^{10} = 2{\cdot}5$ (Lewis, 1942; Crowe, 1955). A similar temperature effect is found in the Rosaceæ and Primulaceæ (Modlibowska, 1945). Tests at 15°, 26°, 30° on the SI species of the Solanaceæ, given in fig. 1, revealed no effect of temperature either in the self-pollen tubes or in the (SI ×SC) pollen tubes. It would appear, therefore, that the effects of temperature on rate of growth and on the inhibition reaction cancelled out in the Solanaceæ. But in the Scrophulariaceæ the temperature experiments were more informative. The means of lengths of pollen tubes in 24 hours are given below:

	Selfed	(SI×SC)
Temperature	*Antirrhinum glutinosum*	*A. glutinosum*×*A. orontium*
15°	1·5 mm.	1·3 mm.
26°	0·05 mm.	0·1 mm.

There is therefore a corresponding effect of temperature on the two types of inhibitions.

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(iii) *Unitary nature of the inhibition*

Perhaps the most remarkable aspect of the (SI ×SC) inhibition is its general nature in contrast to the highly specific nature of self-inhibitions of the (SI ×SI) type. Not only does the (SI ×SC) inhibition occur between widely different species and genera, but any of the different SI alleles in the style have the power to inhibit SC pollen. McGuire and Rick (1954) have shown in *Lycopersicon* crosses that S1.2, S1.c, and S2.c styles all inhibit SC pollen. Mather (1943) found in *Petunia* hybrids that both the S alleles he used from the SI species, when present in the style, either singly, together, or with an SC allele in the F_1, inhibited SC pollen.

This unitary action of the SI alleles is such a widespread phenomenon that it cannot be ignored and must be considered with the other facts bearing on the biochemistry of the incompatibility reaction.

The inhibition of pollen by the style is caused by an endothermic reaction between a specific protein, antigen, in the pollen and an antibody in the style (Linskens, 1953 ; Lewis, 1952). To explain incompatibility in *Petunia*, Straub (1947) has invoked a hypothesis in which an antigen-antibody reaction occurs but the antigen is itself a vital growth substance for the pollen. A modified form of Straub's hypothesis has been adopted by Lewis (1954) and Crowe (1955) to explain interspecific relationship in *Œnothera*.

The (SI ×SC) inhibition can be readily accommodated into this hypothesis if we assume that (1) the S complex is necessary for the production of a pollen enzyme : (2) that by a further activity of the S complex this enzyme is bound to a highly specific protein : (3) that the S gene produces two types of antibodies in the style, a primitive one which is able to combine with the unbound enzyme and a more recent one which combines with the protein of the enzyme-protein complex.

We assume that (1) SC species have only the enzyme without the protein antigen and without the two antibodies in the style. (2) The SI species have the full array of antigens and enzymes. (3) The exceptional self-compatible species, the pollen of which is not inhibited in SI styles, have two stylar antibodies and an altered protein antigen in the pollen. This class (Sc) receives detailed consideration in the following section.

5. THE ORIGIN OF SELF-COMPATIBLE SPECIES

From the data presented in this paper and from arguments which will be developed later, it is assumed with Whitehouse (1950) that self-incompatibility has been established in the angiosperms almost from the time of their origin. The question to ask at this stage is how the self-compatible species existing to-day have originated, and not how self-incompatibility has evolved.

The present results, and the published data in *Nicotiana* species (Anderson and de Winton, 1931) ; *Petunia* species (Mather, 1943) ; *Lycopersicon* species (McGuire and Rick, 1954) and *Antirrhinum* species (Harrison and Darby, 1955) allow the following rule to be formulated :

In the four possible combinations in crossing self-incompatible (SI) and self-compatible (SC) species, pollen-tube growth is uninhibited except in the cross SI style × SC pollen, where it is inhibited similarly to SI pollination.

Naturally where the crosses are very wide there may be other factors obscuring the relationships outlined in the rule. For example these are present sometimes between different families ; as we have shown, the pollen of the Solanaceæ does not germinate on stigmas of *Antirrhinum*. The situation in the Onagraceæ (fig. 3) does not conflict with our thesis, though this family requires special treatment. But even in crosses between closely related species there are several exceptions to the rule which prove to be of great significance.

In the Solanaceæ (fig. 1) our specimens of *Lycium chinense* are self-compatible, and yet its pollen is not inhibited in styles of self-incompatible species. Furthermore its styles inhibit the pollen of the self-compatible species. In *Antirrhinum*, Harrison and Darby (1955) found that the self-compatible *A. majus* was not inhibited in SI species, and its own style rejects pollen of the SC *A. orontium*. Similarly in the Papaveraceæ, the self-compatible *Papaver alpinum* pollen is not inhibited in the styles of the SI, *Papaver nudicaule* (Fabergé, 1944). The pollen of Sc *Nicotiana langsdorfii* is not inhibited in SI *Nicotiana alata* (East, 1919).

We are faced with the four exceptions and others will, no doubt, be found. There is good evidence that all the four species have been SI in the past, and that the change to self-compatibility has occurred recently. *Lycium chinense* has close relatives which are reported to be self-incompatible (East, 1940). It has been grown as an ornamental plant for its berries and is propagated by cuttings. A self-compatible form by mutation would be of great horticultural value and would be selected. *Antirrhinum majus* has been cultivated and selected for self-compatibility. All its wild races are SI (Baur, 1932). Darwin (1876), states that *Papaver alpinum* is self-incompatible, indicating that there are two forms of this species, the Sc presumably being a recent mutant of the self-incompatible form. *Nicotiana langsdorfii* also has a self-incompatible race (Goodspeed, quoted by East, 1940). Thus all these four species have SI races and there is evidence that the Sc condition is a recent acquisition.

Turning to the SC species which conform to the rule we have no evidence that self-incompatible races exist, and there is good evidence in two of the species that they have been self-fertilising for a long time. For example, in the Solanaceæ, *Lycopersicon esculentum* is self-compatible in wild populations (Rick, 1950). This species also

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shows little heterosis and no inbreeding depression. In the Scrophulariaceæ *Antirrhinum orontium* has a small flower which enforces self-pollination. These are differences from other SI species of *Antirrhinum* which could have accumulated only after long self-pollination.*

Our data for the sporophytic system in the Cruciferæ show that pollen tubes are not inhibited in crosses between SI and Sc species whichever way the cross is made, if the Sc parent is from a genus which contains both SI and Sc species. Two genera *Æthionema* and *Draba* in which only SC species have been found (Bateman, 1955) show the (SI × SC) inhibition. Thus there is a parallel with the behaviour found in the gametophytic system. Only those species show the inhibition which have been SC for a long time, as inferred by the occurrence of only SC species in the genus.

The facts indicate the following sequence of events leading to a self-compatible species in families which are primarily and predominantly self-incompatible. A mutation in a self-incompatible species, SI⟶Sc : this is the condition in a recent self-compatible species like *Antirrhinum majus*. A further mutation occurs to give Sc⟶SC : this is found in a long established self-compatible species like *A. orontium* and *Lycopersicon esculentum*.

The genetic evidence for this sequence of mutations is obtained from mutation studies of the S gene in *Œnothera*, *Prunus* and *Trifolium* (Lewis, 1951 ; Pandey, 1956). Spontaneous and induced mutations in pollen-mother-cells give S alleles that are Sc in their action, so that the pollen carrying these alleles is not inhibited in its own style or in styles carrying any of the many normal SI alleles. These mutations occur spontaneously at a frequency of 1×10^{-7}, and if self-pollination occurs they will be rigorously selected. An **SISc** heterozygote is produced, which on further selfing produces homozygotes **ScSc**.

The stylar activity of the **Sc** mutants is unimpaired (Lewis, 1954). A homozygote ScSc produced experimentally in *Œnothera organensis* behaves like the Sc *Antirrhinum majus*. It is self-compatible, its pollen will grow down the style of SI species and its style has the same power of inhibition as the SI styles. Once such an ScSc homozygote became thoroughly established as a self-pollinating species, isolated from its ancestor and near relatives, other changes would occur. Not only will the flower be reduced in size and attraction, having no longer to cater for the pollinating insect, but the self-incompatibility gene will undergo further mutation. The activity of the **S** gene controlling the style reactions is now redundant. Mutations affecting this activity are bound to occur, and without the force of selection against them,

* Information in the literature is in accord with the rule and its exceptions. Kostoff (1941) reports that the pollen tubes of *Lycopersicon esculentum* (SC) are arrested in the styles of *Nicotiana tabacum* (probably Sc) whereas the pollen of *Petunia violacea* SI reaches the ovaries of *Nicotiana glauca*, *N. langsdorfii* (SI and Sc) and *N. suaveolens*. Cooper and Brink, (1940) report pollen tubes of *Petunia violacea* SI and *Lycopersicon esculentum* in the ovaries of *Nicotiana rustica* (SC). *Cf.* Bellartz, *Planta*, 47, 1956.

the mutants will spread in the population. This should give an intermediate stage of a self-compatible species (Sc′) whose style does not inhibit pollen of SC species and whose pollen is not inhibited in SI styles. Such an intermediate species has not been found.

So far the mutations postulated have been observed in experiment ; the remainder is conjecture.

The final stage as found in the old SC species would be the result of a third mutation of the **S** gene, leaving the pollen unprotected from the inhibitory effect of the SI style. The second mutation, Sc⟶Sc′, would have to be well established in homozygous individuals before this last mutant could survive. This three-stage sequence of events, two steps of which are known from experiment, is somewhat elaborate. But after it has been postulated it is obvious that these are the minimum steps required. Clearly a mutation in an SI species in one step to SC could not become established. A pollen grain carrying such a mutant would be barred from fertilisation because there would be no style in which it could grow. Even if such a mutant occurred in an embryo-sac, there would be no selection for it, and furthermore a plant arising from it would be a heterozygote, **SI.SC**. This would be self-incompatible (McGuire and Rick, 1954 ; Harrison and Darby, 1955) with the result that homozygotes could not be formed. There is a small chance of it arising in an embryo-sac followed by non-reduction and the production of an apomictic homozygote **SCSC**. This would have little chance of survival because, as an isolated individual, it would be deprived of the interchange of genes by outcrossing at the crucial early stages of evolution to SC. There is good evidence that one-step mutations from SI to SC do occur but are lethal. For example in the tetraploid species *Trifolium repens*, Pandey (1956) finds that mutations which lack all detectable activities, stylar and pollen, of the **S** gene occur frequently. But these are not recovered in diploid species. It is presumed that a locus, no longer directly concerned with incompatibility, is able to mask the lethal effect of a deletion at the S locus in this tetraploid.

Once a species has self-pollination enforced upon it by lack of a pollinating insect then there would be a great selective pressure to follow the sequence of events SI⟶Sc⟶Sc′⟶SC.

6. BALANCED LETHALS AND INCOMPATIBILITY IN *ŒNOTHERA*

The great similarity of the incompatibility systems in the Solanaceæ, Scrophulariaceæ, and Onagraceæ gives us some grounds for applying the conclusions derived from the data in the Solanaceæ and Scrophulariaceæ to the Onagraceæ.

From the data in the Solanaceæ and Scrophulariaceæ we have concluded that Sc species which are not inhibited as pollen in SI styles have only recently become Sc. The results of all interspecific

pollinations in *Œnothera* show no inhibition of Sc pollen in SI styles. These pollinations include the ring-forming species *O. biennis* and *O. lamarckiana*, so we conclude that these species have been SI in the past and have not progressed very far towards a full SC species. But the elaborate system of balanced lethals and ring chromosomes accompanied by cleistogamy would suggest that self-pollination has been established over a long period (Cleland, 1950 ; Darlington, 1931). This seeming paradox, an old self-pollinating species retaining the vestiges of self-incompatibility which allow its pollen to grow down an SI style, can readily be resolved if we assume that the SI system has played a key role in the evolution of the balanced lethal system.

Before doing this we should point out the main weakness of our data in the Onagraceæ. There is no clear-cut (SI $\times$SC) inhibition in any crosses even when *Epilobium* and *Godetia* pollen are used. These are genera without a regular ring-chromosome mechanism and without incompatibility. The data would be more compelling if some of these had shown (SI $\times$SC) inhibition.

The ring-forming, self-pollinating species with balanced lethals have evolved from ancestors with seven bivalents and cross-pollination. Steiner (1956) presents good evidence that the alpha complex of *O. biennis* contains an incompatibility allele while the beta complex does not carry such an allele. In the ring-forming species there may be balanced lethals in other chromosomes, but the key pair is the one which has the pollen lethal. Catcheside's (1936) observations from trisomics indicate that only one or two chromosomes contain lethals. Our hypothesis is that the pollen lethal was the first to arise and was present as the incompatibility gene in all plants of the cross-pollinating ancestor. This is similar to Crosby's (1954) and Steiner's (1956) hypotheses.

There are two known ways in which a balanced lethal system could start from self-incompatibility and both these ways have their counterpart in modern ring-forming *Œnotheras*.

(i) *Sc mutation*

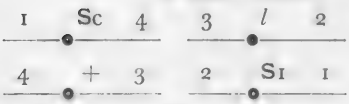
First a mutation of S1$\longrightarrow$Sc similar to the mutation found in experiments with *Œnothera organensis* would be constantly occurring in an SI ancestor. If cross-pollination was reduced by the lack of a pollinating insect then this mutant would be selected rigorously. The homozygote ScSc would be viable at first, and the population would consist of groups of plants, ScSc, ScS1 : ScSc, ScS2, etc. All that is required to complete the first stage is a reciprocal interchange in one arm of the Sc chromosome with another non-homologous chromosome, accompanied by either a position effect, gene mutation or minute deletion that is lethal in the homozygote or in the egg.

The steps in the evolution of the system would be :

1. S_1S_2 —mutation→ S_1Sc —population→ S_1Sc, $ScSc$, S_2Sc

2. Interchange of chromosome 1 Sc 2
   with chromosome 3 4

A chain of four

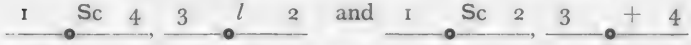


1 Sc 4 3 *l* 2 — transmitted on selfing only in pollen

4 + 3 2 S_1 1 — transmitted only in egg

3. Further interchanges may occur in other chromosomes ending in a ring of 14.

If the lethal, *l*, is a zygotic lethal we could have complexes :



1 Sc 4, 3 *l* 2 and 1 Sc 2, 3 + 4

These would segregate regularly to give functional pollen and eggs of both complexes, but only the heterozygotes survive as in *Œnothera lamarckiana* (Renner, 1914).

The pollen-egg lethal system would be :

1 Sc 4, 3 *l* 2 and 1 S_1 2, 3 + 4

This is found in many species including *O. biennis* (*cf.* Cleland, 1950).

One essential feature of this origin is that the first interchanged chromosome should involve the mutant Sc allele and not the S_1 allele. Cleland (1950) in his detailed analysis of the interchange in N. American *Œnotheras* states that " none of the alpha (pollen lethal, *i.e.* S_1) have suffered the interchange 1.2 3.4⟶1·4 3·2 whereas all the beta (egg lethal, *i.e.* Sc) complexes have done so ".

Cleland (*loc. cit.*) is also impressed by the narrow range of interchanges found in a wide sample of material. He states " The presence of so many complexes with similar segmental arrangements constitutes, therefore, a fact of real phylogenetic significance. It seems to indicate that these complexes are more or less closely related and that a considerable proportion of the complexes existing throughout the range of *Œnothera* have not departed very far from the ancestral segmental arrangement from which they have been derived." If, as we believe, the first important interchange in all cases involved the Sc locus then this narrow range of complexes is not unexpected.

One puzzling feature of the original balanced lethal system of the ring-forming *Œnotheras* in which the lethals are gametophytic is that these " lethal " pollen grains are often viable in outcrosses. The balanced lethal system working on pollen gametophytes cannot

satisfactorily explain how these gametophytes are viable when they are on another female. The explanation for this is to assume, as Steiner (1956) has done for *O. biennis* that different heterozygotes (species) have different SI alleles. For example in the cross between S1Sc × S2Sc, the S2 pollen, which is "lethal" on selfing, can function normally.

An unexplained but extremely important phenomenon was found in the autotetraploid of *O. pictorubata* by Renner (1939). The diploid is a self-pollinating ring-forming heterozygote : the tetraploid is self-incompatible, setting no seeds. The tetraploid is fertile when used as a female or a male with other tetraploid heterozygotes and as a female with its own diploid. It was sterile when used as a pollen parent on to its diploid form. Clearly the cause of the self-incompatibility was not because of sterile pollen or eggs, but because the pollen would not function when selfed, and the cause was actually found to be due to inhibition of pollen tubes. It was also found that the viable pollen grains of the tetraploid contained both the egg and pollen complexes.

This unusual phenomenon of self-incompatibility in an autotetraploid of a diploid Sc species is in agreement with our hypothesis.

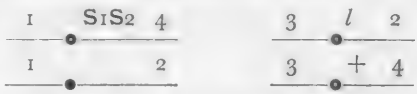
If the pollen "lethal" in the diploid were a recessive lethal causing a deficiency in the pollen, as the balanced lethal theory implies, then the diploid pollen of the tetraploid with both complexes should be able to function in selfing because there would be the dominant wild-type gene to cover the deficiency. But the results with the diploid pollen show that the lack of pollen-tube growth is not due to a deficiency but to a positive inhibition which cannot be rectified by the gene which allows pollen-tube growth in the haploid grain. Clearly, if we assume the diploid *O. pictorubata* is ScS1, then diploid pollen grains of the tetraploid, which are viable, will be ScS1 and these will be inhibited in ScSc S1S1 styles of the tetraploid or ScS1 styles of the diploid but will not be inhibited in ScSc S2S2 styles of another tetraploid heterozygote.

(ii) S_1S_2 *duplication*

The second way of starting a pollen lethal system from the S gene is by unequal crossing-over to give a duplication of the S locus in one chromosome and a deficiency in the other. This has consequences different to those of the first method of Sc mutation. Catcheside (1947) has described such an interchromosomal duplication and deficiency that arises frequently in *Œnothera blandina*. Indeed, Darlington (1931) postulated an interchromosomal duplication in two chromosomes and a deficiency of a small non-lethal interstitial segment in the two homologous chromosomes as a means of obtaining viable segmental interchanges in *Œnothera*. Duplication to give an adjacent repetition of the same chromosomal material is common in *Drosophila* (Lewis, E. B., 1951).

In an SI species two S genes in one chromosome and none in the other would have two effects. The chromosome with the two S genes in an SI species would be S_1S_2. In diploid pollen grains of tetraploid *Œnothera organensis* and in several other genera (*cf.* Lewis, 1954) competition between two different S alleles occurs so that neither allele can operate. Hence by analogy we would expect a similar situation to occur in haploid pollen grains carrying different S alleles in the same chromosome, in which event $S_{1.2}$ pollen would be compatible on styles carrying S_1 and S_2 and on all other styles. The S_1S_2 duplication would then be the starting point of the pollen transmissible complex. The S deficiency, on the other hand, would be lethal in the pollen. That S gene deficiencies are lethal in pollen has been found from mutation experiments in *Œnothera organensis*, *Prunus avium* and *Trifolium* species (Lewis, 1954 ; Pandey, 1956).

Thus, with the necessary translocation, we have :



The classical example of this type of complex is found in *Œnothera muricata* in which half the pollen is inviable (Renner, 1919). Professor Cleland has drawn our attention to *O. chicagnensis* which has normal pollen but one complex is never transmitted through the style.

Test of the hypothesis—general evidence

There are several tests for proving or disproving these two ways of origin of the balanced lethal complexes. A detailed study of pollen-tube growth in controlled self- and cross-pollinations of well chosen genotypes would be informative. The growth of incompatible pollen tubes in SI species of *Œnothera* has a characteristic temperature curve (Lewis, 1942 ; Crowe, 1955). If a similar temperature curve was found in the pollen carrying the lethal complex, then there could be little doubt that the same incompatibility reaction and probably the same S gene is operating.

The behaviour of autotetraploids could be a valuable indication. In species such as *O. lamarckiana*, with zygotic lethals and a supposed ScSc constitution, the autotetraploid should be self-compatible. In fact the autotetraploid *O. lamarckiana gigas*, which has zygotic lethals, is self-compatible. In species which have gametophytic lethals and in which the pollen can function in some outcrosses such as *O. biennis*, *O. pictorubata*, etc. then we might expect self-incompatibility of the autotetraploid. However, if the Sc chromosome in the duplex state, ScSc, is viable in the pollen then the tetraploid would be self-compatible. In such species as *Œnothera muricata* with a supposed S_1S_2 duplication with competitive interaction of the S alleles, then the pollen from the tetraploid should remain self-compatible.

Darlington and Gairdner (1937) have made a comprehensive comparison of the ring-forming species in plant genera and note that "the self-sterility of *Campanula* while promoting the occurrence of interchange heterozygotes at the beginning, is not likely to favour the further development of a multiple ring system for this reason: self-sterility has already given the species a high hybridity equilibrium which a multiple ring system can do little to increase". But if self-pollination became a necessity because of difficulty of ensuring cross-pollination then there would be a great advantage of initial self-incompatibility not only in forwarding the interchange but in preserving it later when the self-incompatibility had broken down by the Sc mutation or by the S1S2 duplication. It is significant that the three species *Zea mays*, *Pisum sativum* and *Datura stramonium* which have interchange ring chromosomes in experiment but not in the wild are from exclusively SC genera.

It is also suggestive that the two other examples of ring-forming heterozygotes species are *Rhœo discolor*, which has the SI genus, *Tradescantia*, as a near relative, and *Hypericum punctatum* (Hoare, 1931) which has species in the same genus which are SI (East, 1940).

The important feature of the Sc origin of the balanced lethals is that a ready-made pollen lethal was there in the cross-pollinated ancestor, and only the simplest structural change with the assistance of enforced self-pollination was necessary to get the system launched. Clearly the final success of the change depends upon all the factors which have been expounded by Darlington (1936), such as terminal chiasmata and regular orientation of centromeres.

The paradox, referred to earlier, that the ring-forming *Œnothera* have been self-pollinated for a long time and yet our results on interspecific pollination indicate that they are behaving like species which have only recently mutated from self-incompatibility, is now resolved. Clearly if the SI gene is the key to the balanced lethal system, then it must retain the activity revealed by the interspecific pollinations.

7. ADAPTATION AND THE BREEDING SYSTEM

Darwin proved the advantages of outbreeding, and it has often been demonstrated since in a wide variety of organisms. We do not intend to add to this list but wish to make two points.

1. The evolution of many complex outbreeding mechanisms in flowering plants, and also in bacteria, fungi, algæ and animals, emphasises their extreme importance in the evolution of organic life. The recent discoveries of sporophytic systems in the angiosperms are only more examples of the lengths an organism will go to in its pursuit of hybridity (*cf.* Darlington, 1939).

2. In the flowering plants and fungi, and possibly in other groups, where great diversity of outbreeding devices has been found, a high

proportion of highly successful species exist which have abandoned outbreeding and have had sexual self-reproduction for a long time.

Whitehouse (1950) has actually attributed the explosive flowering of the angiosperms in the Cretaceous period to the evolution of the style and self-incompatibility. Undoubtedly self-incompatibility, particularly of the multiple-allelic type, is one of the most efficient outbreeding mechanisms that can be envisaged. Our evidence presented in this paper proves that self-incompatibility systems are not recent innovations in the angiosperms. There is only one conclusion to be drawn from the results of crosses within and between the two families, Solanaceæ and Scrophulariaceæ. The incompatibility system was fully evolved in the common ancestor of these two large families.

One other conclusion which seems highly probable from our data is that the self-compatible species in families which have self-incompatibility have been derived by a degradation process from self-incompatible ancestors.

The paradox of evolution is the prime importance of outbreeding on the one hand and the presence of a high proportion of long established and successful species that are inbreeders. It has frequently been said that inbreeding may lead to an immediate advantage at the expense of long-term fitness. This is true, but it is not the whole answer to the paradox, because our evidence shows that many plants have been inbreeders for a long time and have yet maintained their fitness. The answer to this may be found in what we may call the *critical stage in evolution*. In the life of an organism, whether it is a self-reproducing macromolecule in a primitive sea or a higher plant or animal, there is a period in its history when it is competing in a common environment with other organisms. This is the period when an outbreeding mechanism is absolutely essential for survival. It is only by an outbreeding system that the immense advantage of gene recombination can be obtained. This period is probably short as implied by Gause's (1934) principle, that two or more forms with identical ecological requirements cannot co-exist indefinitely in the same environment. After the species has captured its environment and the critical stage is over it may still retain its outbreeding system, or because of some advantage or because it is forced to by causes outside its own control, such as the lack of a pollinator, it may abandon the very means of its success. Only after a cataclysmic change in the environment which might not occur for a million years will the inbreeder be at a disadvantage.

8. SUMMARY

1. It is argued that the distribution of the five self-incompatibility systems in the families of the angiosperms is evidence that the systems were perfected at an early stage in the evolution of the group.

2. The value and shortcomings of interspecific crosses to reveal similarities and differences in breeding systems are outlined.

3. In the gametophytic system the literature reveals four examples of pollen inhibition of a self-compatible (SC) species in the styles of a self-incompatible (SI) species. This is the (SI × SC) inhibition. But at the same time four examples are quoted where such interspecific crosses do not show the inhibition.

4. Pollinations between different SI and SC species and genera in the Solanaceæ and between this family and the Scrophulariaceæ prove that the (SI × SC) inhibition rule is a general phenomenon.

5. The exceptions to the rule are varieties and species (Sc), which have only recently mutated from an SI species. The species which obey the rule have also originated from an SI ancestor but a long time ago.

6. A probable sequence of mutations, some of which have been produced experimentally, is postulated to explain the occurrence and behaviour of the three types of species SI, Sc (recent), SC (old).

7. Pollinations between species and genera in the Cruciferæ with a sporophytic system, and examples culled from the literature, indicate that the (SI × SC) rule may hold in sporophytic systems.

8. Interspecific and intergeneric pollinations in the Onagraceæ lead to the conclusion that the ring-forming, self-pollinating *Œnotheras* behave like recent Sc species. A theory of the origin of the permanent heterozygote and balanced lethals from the incompatibility gene is given: (1) by mutation SI—→Sc or (2) by a duplication and deficiency to give an S_1S_2 duplication and an S deficient chromosome.

9. The (SI × SC) inhibition is discussed in relation to the biochemistry of incompatibility and the " critical stage in evolution ".

9. APPENDIX, SI . SC . Sc

The symbol SI is used for a self-incompatible species.

The symbol SC is used for an old self-compatible species which shows (SI × SC) inhibition.

The symbol Sc is used for a self-compatible species which does not show the (SI × SC) inhibition. In most cases such species have a recent history of self-incompatibility.

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RECURRENT CHANGES IN COLONY MORPHOLOGY DEPENDENT ON INTRACOLONY SELECTION OF PROTOTROPHS OCCURRING IN RED YEAST *

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1. INTRODUCTION

CERTAIN genetic variants that occur in red, adenine-requiring strains of *Saccharomyces cerevisiæ* are readily detected on the basis of colour differences. Instances of such genetic instability in these red yeasts have been reported by Lindegren and Lindegren (1947), by Ephrussi, Hottinguer, and Tavlitzki (1949), and by Roman (1956). The phenomenon reported in this paper was first observed by the writer in the course of experiments in which red yeast had been plated upon media containing different concentrations of sugar. The colonies growing upon 0·5 per cent. glucose medium were observed for the most part to be homogeneous in appearance, and true to type for the culture (fig. 1). On the 5·0 per cent. glucose medium, a large number of the red colonies were partly or completely masked by white yeast growing from them (fig. 2). The white yeast tended to occur in irregular but approximately spherical masses. In general appearance, these plates suggested pans of partially popped, red-kerneled popcorn. The results of experiments reported here indicate that this phenomenon in red yeast depends upon a particularly strong selective advantage of spontaneous, adenine-independent mutants under particular conditions of culture.

2. MATERIALS AND METHODS

The red, adenine-requiring cultures with which these experiments were done were vegetative transfers from haploid strains 173/1*a*, 173/1*b*, and 174/1*b* (Ephrussi, Hottinguer, and Tavlitzki, 1949). The mutant gene in these strains, to which the adenine deficiency and red pigment are referable, has been designated as *ad 2* by Roman (1956). The greater part of the work was done with clones C-34 and C-33, derived respectively from single cells isolated out of strains 173/1*a* and 173/1*b*. Strain C-34 was previously described under the label PC-34 (Srb, 1956), but is here redesignated to avoid confusion with "petite colonie" mutants, sometimes abbreviated as PC. Inocula for plating experiments were grown for 20 to 48 hours in standing liquid culture. The liquid yeast extract medium and the solid yeast extract medium used in plates were basically those described by Leupold and Hottinguer (1954), except that glucose was added to the plating media in various concentrations. Bacto Yeast Morphology Agar was used as a chemically defined plating medium, and either was or was not supplemented with adenine-sulphate and/or glucose according to the purposes of an experiment. Standard phosphate buffers were used to control *p*H of the media for certain experiments. Inocula onto plates were made out of suspensions of cells in Ringer's solution, and were spread with a glass spreader. All incubations were at 24°-26° C. Determinations of *p*H were made with a glass-electrode. Estimations of cell numbers were

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made on the basis of hemocytometer counts or on the basis of counts of colonies derived from cells plated through dilution series. The beta-propiolactone used for mutagenic purposes was kindly supplied by the B. F. Goodrich Company.

3. RESULTS

(a) *Primary observations*

The primary observations leading to the experiments described here were as follows. Cells of red, adenine-requiring strain 173/1*b* had been spread on yeast extract agar plates supplemented with glucose at two different levels, 0·5 and 5·0 per cent. After six days' incubation the plates were opened. On the medium with 5·0 per cent. sugar, no purely white colonies were observed but many of the red colonies showed more or less massive eruptions of white yeast. These eruptions, which henceforward will be called *bursts*, were frequently six or more times the height of the red colonies from which they arose or of the height of red colonies not showing bursts. Usually the general outline of well-developed bursts was roughly spherical. Most frequently, bursts arose from near the centre of the red colonies. However, bursts also arose near the peripheries of the red colonies. Large colonies frequently showed more than one burst, with, in general, the largest burst being near the centre of the colony and the others being smaller and peripheral. Except in the case of very small colonies showing bursts, even the peripheral bursts seldom spilled over onto the medium surrounding the colonies bearing them. Much of the growth of the bursts appeared to be vertical in direction. Cultures established from cells isolated from the bursts proved to be adenine-independent when plated upon Yeast Morphology Agar, and gave rise to white colonies.

On the medium containing 0·5 per cent. sugar, no bursts were observed. Approximately 1 per cent. of the colonies were small in size and were either non-pigmented or were brown rather than red. On the basis of work reported by Tavlitzki (1951), these exceptional colonies were thought possibly to be adenine-requiring "littles", which form red pigment only when glucose is in excess and the cells have ceased proliferating because of lack of adenine. Cultures established from the exceptional colonies were found in fact still to be adenine-requiring; spectroscopic analyses, confirmed by Professor B. Ephrussi, showed a sample of these cultures to have the cytochrome deficiency typical of little yeast. Certain of the red colonies showed sectors, in some instances colourless, and in other instances coloured other than red. The sectors were in part of the typical wedge-shaped kind, flaring out towards the edge of the colony, but other sectors appeared as flecks or dots of differently coloured yeast within a red colony. Cultures established from isolates out of 40 of these sectors proved in the vast majority of instances to be adenine-requirers, which were either "little" yeast or were "big" yeast, showing a colour variation from the typical red of the original adenine-requiring

strain. Two of the culture isolates, deriving in each instance from a pick from a white dot sector, were found to consist of adenine-independent, non-pigmented cells.

(b) *Effects of sugar concentration, of pH, and of adenine*

The frequency of bursts in relation to sugar concentration in the medium was studied in several red, adenine-requiring strains of yeast. Results of such an experiment are shown in table 1. For

TABLE 1

Effect of glucose concentration on occurrence of bursts in adenine-requiring strains of yeast. Basic medium was yeast extract medium. Each value is based on the count from 4 plates

	Per cent. glucose					
	10	8	5	3	1	0·5
Strain 173/1*b*.						
Total colonies . .	181	162	184	198	175	179
Total colonies with bursts ·	46	83	60	25	0	0
Total bursts . . .	61	122	73	28	0	0
Per cent. colonies with bursts	25	51	33	13	0	0
Strain C-33						
Total colonies . .	147	229	159	163	163	217
Total colonies with bursts ·	53	88	61	34	0	0
Total bursts . . .	77	118	75	36	0	0
Per cent. colonies with bursts	36	38	38	21	0	0
Strain 174/1*b*.						
Total colonies . .	221	272	228	256	252	233
Total colonies with bursts ·	79	97	96	106	0	0
Total bursts . . .	100	128	140	150	0	0
Per cent. colonies with bursts	36	36	42	41	0	0
Strain C-34						
Total colonies . .	221	225	198	173	162	220
Total colonies with bursts ·	76	75	92	58	0	0
Total bursts . . .	102	93	124	69	0	0
Per cent. colonies with bursts	34	33	47	34	0	0

each strain at each sugar level the values represent the total of counts from 4 plates, inoculated with a suspension of cells diluted so as to give approximately 50 colonies per plate. The medium was yeast extract medium. The data are consistent in that none of the strains shows bursts at 1·0 or 0·5 per cent. glucose, while all the strains show bursts at 3·0 per cent. glucose and above. The data perhaps suggest that the yeast strains differ among themselves, with reference to frequency of bursts at glucose levels of 3·0 per cent. and above. The number of observations, however, is insufficient to permit firm conclusions as to possible differences among these strains.

Since differences in sugar concentration might be supposed perhaps to be reflected in differences in *p*H of the media, the *p*H of yeast extract media on which colonies were growing was tested. For media

supplemented with 5·0 per cent. glucose the *p*H was found to be 4·5; for media supplemented with 0·5 per cent. glucose, the *p*H was found to be 5·0. Therefore, experiments were done in which the frequency of bursts was observed on media containing 5·0 or 0·5 per cent. glucose, unbuffered or buffered to different *p*H levels. The results of such an experiment with strain C-34 are shown in table 2. The data clearly show that, among the *p*H levels at which observations were made, bursts do not occur at *p*H levels above 4·5, irrespective of sugar concentration. Bursts do occur at *p*H 4·5, also

TABLE 2

Effect of p*H of medium on the occurrence of bursts. Six plates per treatment. All plates inoculated from the same culture of strain C-34, diluted to provide an estimated 15-20 colonies per plate*

Medium	Mean number colonies per plate with		
	0 bursts	1 burst	more than 1 burst
Yeast extract (5 per cent. glucose)			
Unbuffered (*p*H 4·5)	3·0	4·3	6·8
Buffered, *p*H 6·5	14·3	0·0	0·0
Buffered, *p*H 5·3	15·2	0·0	0·0
Buffered, *p*H 4·5	10·2	3·7	2·0
Yeast extract (0·5 per cent. glucose)			
Unbuffered (*p*H 5·0)	15·9	0·0	0·0
Buffered, *p*H 6·7	15·0	0·0	0·0
Buffered, *p*H 5·3	15·5	0·0	0·0
Buffered, *p*H 4·5	10·7	1·4	0·2

irrespective of sugar concentration. As between sugar concentration and *p*H, then, the controlling factor for appearance of bursts is primarily *p*H.

At appropriate sugar levels, bursts appeared when strain C-34 was grown on Yeast Morphology Agar supplemented with 10 mg. per litre of adenine-sulphate. Therefore, the complex mixture of substances found in yeast extract medium is not essential for the occurrence of bursts in this strain of red yeast. The frequency of bursts in relation to adenine concentration was then studied in strains C-33 and C-34. The basic medium for these experiments was Difco Yeast Morphology Agar, buffered to *p*H 4·7 and supplemented with 5 per cent. glucose. The results of a typical experiment of this kind, summarised in table 3, show that with increasing concentration of adenine the bursts become less prominent, although not necessarily less frequent, and finally disappear. At a concentration of 10 mg. adenine-sulphate per litre, typical bursts appeared. At 20 mg. per litre, no well-developed bursts were observed, but approximately 10 per cent. of the red colonies showed slightly protuberant white sectors. Isolates made from these sectors yielded cultures of white,

adenine-independent cells. At 40 and 80 mg. per litre, typical bursts did not appear, and neither did protuberant sectors of the kind just described. Instead, certain of the red colonies showed minute sectors appearing as white dots among the red yeast. Again, isolates made from these dot sectors yielded cultures of white, adenine-independent cells. There seems no reason to doubt that the cells found in bursts, in the protuberant sectors, or in the dot sectors are basically the same. At 160 mg. per litre of adenine-sulphate, no sectors of any kind could be detected. However, conditions for detecting sectors on the basis

TABLE 3

Effect of adenine concentration on occurrence of bursts. Basal medium Difco Yeast Morphology Agar, supplemented with 5 per cent. glucose. Each value based on counts of 3 plates, with 150 to 200 colonies per plate

Mg. per litre adenine-sulphate	Strain	Percentage of colonies with slightly protuberant		
		bursts	sectors	dot sectors
10	C-34	4·6	0·0	0·0
	C-33	7·0	0·0	0·0
20	C-34	0·0	8·7	0·0
	C-33	0·0	11·4	0·0
40	C-34	0·0	0·0	7·1
	C-33	0·0	0·0	5·0
80	C-34	0·0	0·0	0·5
	C-33	0·0	0·0	0·4
160	C-34	0·0	0·0	0·0
	C-33	0·0	0·0	0·0

of colour difference were poor. At this highest concentration of adenine, colonies developed pigment very slowly indeed. Eight days after inoculation of the plates the colonies were colourless, and at 10 days the colony colour was only light pink. Inspection of the colonies revealed neither bursts nor protuberances suggesting bursts. It should be said that with each increase in adenine concentration, the average size of colonies deriving from cells of strains C-33 and C-34 was larger. Therefore, well-developed bursts occurred only where adenine concentration was limiting for the growth of these strains.

(c) *Experiments on mutation and selection*

The occurrence of white, adenine-independent cells (prototrophs) within colonies of red, adenine-dependent yeast appears to be a mutational phenomenon. Since the mutant cells were manifest only under particular cultural conditions, the question arose as to whether conditions favourable to the appearance of bursts acted by influencing the frequency of mutation or by providing selective advantage to

spontaneously occurring adenine-independent cells. This issue was explored in several ways.

Standard back-mutation techniques were employed to test the possible effects of treatment of cells of strain C-34 with high *v.* low concentration of glucose, with high *v.* low concentration of adenine, and with high *v.* low *p*H. No significant differences in frequency of appearance of adenine-independent mutants were found to result from these various treatments. However, strain C-34 has particular disadvantages for back-mutation studies (Srb, 1956), and less reliance has been placed on these results than on those from the studies next to be described.

If the burst phenomenon depends upon conditions of intracolony selective advantage, rather than depending upon altered mutation frequency, colonies on 0·5 per cent. glucose medium might be expected to include adenine-independent cells even though bursts do not appear. This proposition was tested in the following manner. Cells from strain C-34 were inoculated onto yeast extract plates either containing 0·5 or 5·0 per cent. glucose. As usual, the red colonies on the 0·5 per cent. plates did not develop bursts ; a high frequency of colonies on the 5·0 per cent. plates showed bursts. Whole colonies from the 0·5 per cent. plates were scraped up separately with a small spatula, and the cells of each colony were suspended in a small volume of Ringer's solution. The cells from each colony were then spread onto a separate plate of Yeast Morphology Agar not supplemented with adenine, where each prototrophic cell might be expected to form a white colony and where adenine-dependent cells would not grow. The results, summarised in table 4, indicate that certain colonies not showing bursts must in fact have contained adenine-independent cells. From some of the red colonies, the number of derived adenine-independent colonies was very large. It appears, then, that proliferation of adenine-independent cells may occur within adenine-dependent colonies, even though this proliferation is never so extensive as to result in a burst.

Direct tests were employed to see whether colonies of strain C-34 grown on media with supplements of 0·5 or of 5·0 per cent. glucose are differently favourable for the proliferation of adenine-independent cells. Suspensions of cells of strain C-34, calculated to give about 20 colonies per plate, were spread on yeast extract medium containing either 5·0 or 0·5 per cent. glucose. After 3 days, when colonies had grown to appreciable size, and before spontaneous bursts had appeared on the 5·0 per cent. glucose plates, the centres of colonies on both kinds of plates were inoculated with white, adenine-independent cells. The inoculations were accomplished as direct transfers from bursts, made with a fine platinum needle. In all instances on the 5·0 per cent. glucose plates, the inocula proliferated immediately, and in such fashion as to resemble spontaneous bursts (fig. 3). Inocula into colonies on the 0·5 per cent. glucose plates did not proliferate greatly. At a

time when bursts were well developed on the 5·0 per cent. glucose plates, inoculated colonies on the 0·5 per cent. plates showed only small white sectors at the points of inocula. These sectors were not elevated above the rest of the surface of their colonies.

Similar results were obtained from an experiment that was identically designed except that the inoculum into colonies was with a prototrophic mutant that had been isolated from a burst and then subcultured many times. Similar results were also obtained when colonies were inoculated with cells of strain 174/1*d*, a standard adenine-independent strain not derived as a mutant out of an adenine-requiring culture. This latter observation indicates that the growth properties

TABLE 4

Presence of prototrophic cells in colonies not showing bursts. Each value indicates the number of white, adenine-independent colonies appearing when all the cells of a colony of strain C-34 were spread on Yeast Morphology Agar unsupplemented with adenine. Colonies of strain C-34 were grown on 0·5 per cent. glucose yeast extract medium, were red coloured, and had not shown bursts

Colony A	0	Colony K	47
B	0	L	1
C	63	M	8
D	1	N	0
E	7	O	*ca.* 950
F	61	P	12
G	0	Q	1
H	0	R	*ca.* 400
I	10	S	3
J	153	T	76

of prototrophic cells in bursts are not peculiar to revertants occurring in adenine-dependent strains. The results of the entire experimental series are consistent in indicating that colonies grown under the conditions found in unbuffered 5·0 per cent. glucose plates provide a favourable environment for the rapid proliferation of adenine-independent cells. The conditions in colonies grown in unbuffered 0·5 per cent. glucose plates do not favour the proliferation of adenine-independent cells.

Another kind of experiment was utilised to measure the probability, under appropriate conditions, of an adenine-independent cell expressing itself as a burst in a colony of red yeast. The technique involved the use of giant colonies (Winge and Laustsen, 1937) whose original point inocula consisted of many cells. Known numbers of cells of strain C-34 and of adenine-independent cells from a culture derived out of a burst were suspended together in Ringer's solution. One-tenth ml. inocula from this suspension were then placed as single unspread drops either onto 5·0 per cent. glucose or 0·5 per cent. glucose yeast extract medium. For controls, similar inocula were made from suspensions consisting of the same frequency of cells from strain C-34 but not mixed with adenine-independent cells. The giant colonies formed on 0·5 per cent. glucose did not produce bursts,

whether or not the inoculum contained added prototrophs. The giant colonies formed on 5·0 per cent. glucose did form bursts. Comparison of the frequency of bursts from colonies initiated with the mixed suspension as against the frequency of bursts from colonies initiated only with C-34 cells gave an indication of the probability of *recovery as bursts* of the adenine-independent cells present in the inocula consisting of the mixed suspension.

Table 5 summarises the results of one experiment of this kind. For the giant colonies derived from a mixed suspension, the single drop inocula were calculated each to contain approximately 65×10^6 red, adenine-requiring cells from strain C-34 and 100 adenine-independent cells. To check the numbers of adenine-independent cells, drop inocula of the same kind were spread on Yeast Morphology Agar, where the only colonies growing would be those deriving from the prototrophs. The average number of white, adenine-independent colonies appearing on 6 plates of this kind was 83. The control giant colonies were derived from unspread drop inocula simply containing approximately 65×10^6 cells from strain C-34.

Table 5 shows that no bursts appeared in giant colonies grown on the 0·5 per cent. glucose medium. On 5·0 per cent. glucose, the number of bursts was quite variable from colony to colony, both among colonies constructed out of mixed inocula and among colonies where only spontaneous bursts could be expected. However, the number of bursts was always substantially larger for giant colonies derived from mixed inocula. Comparing giant colonies from mixed inocula with control colonies, the average difference in bursts per colony is 72. Since about 83 adenine-independent cells were put into each of the mixed colonies, the probability of recovery of such a cell as a burst would appear to have been high. In fact, the data may provide an underestimate of this probability of recovery. Bursts occurring in the giant colonies were often very near one another, so that with time neighbouring bursts fused. It seems probable that certain apparently single bursts may have had more than one point of origin from within the colony.

A number of additional experiments with giant colonies gave essentially similar results. This experimental series confirms that at low *p*H and with appropriate adenine concentration prototrophic cells occurring within colonies of red, adenine-requiring yeast have an enormous selective advantage. The advantage is so great that almost any prototrophic cell, occurring independently within the colony, has a high probability of expressing itself as a burst.

(*d*) *The genetics of prototrophs isolated from bursts*

Relatively little is known about the genetics of adenine-independent cells that occur in the bursts. The results of studies that have been made are consistent with the supposition that prototrophs occurring

in the bursts are revertants at the locus of the gene that determines simultaneously red colour and adenine requirement. A prototrophic culture isolated from a burst in a colony of strain C-33 was crossed with strain 173/1*a*. Twelve isolated zygotes reproduced as clones that were all white and adenine-independent. Diploid cells were then allowed to sporulate, and the ascospores were isolated from individual asci. All four spores germinated from 12 of the 15 asci

TABLE 5

Occurrence of bursts in artificially constructed giant colonies. Basal medium yeast extract. Counts of bursts made after 5 days. Inoculum for each artificially constructed colony was about 65×10^6 cells of strain C-34 and 83 prototrophic cells. Inoculum for each control colony was about 65×10^6 cells of strain C-34

	Number of bursts	
	Artificially constructed colonies	Control colonies
0·5 per cent. glucose		
Colony A . . .	0*	0*
Colony B . . .	0*	0*
Colony C . . .	0*	0*
5·0 per cent. glucose		
Colony A . . .	124	30
Colony B . . .	104	39
Colony C . . .	108	50
Mean . . .	112	40
Mean difference : 112—40 = 72		

* White dot sectors but no bursts.

dissected. In each instance, the ascus gave a 2 : 2 segregation for the alternatives adenine dependence and adenine independence. A second cross was made with a prototrophic culture isolated from a burst in a colony of strain C-34, the other parent being strain 173/1*b*, red, adenine-requiring. Ten zygotes provided white, adenine-independent clones. Of the 27 asci that were dissected, 20 gave spores all 4 of which germinated. In 19 of these 20 instances a clear 2 : 2 ratio was observed. In one instance, the germinated spores on first test appeared to give rise to clones 3 of which were prototrophs and 1 of which was a red adenine-requirer. On retest, it was found that one of the 3 clones of white prototrophs was a mixed culture, containing a small proportion of red, adenine-requiring cells. A reasonable possibility exists that the mixed culture represents an instance of back-mutation occurring fairly early in the history of a clone that was originally adenine-requiring. In any case, no critical evidence exists to show that the prototrophs found in the bursts were something other than back-mutants. The results, in fact, are basically

similar to those reported (Srb, 1956) for a cross involving one of the adenine-requiring strains (173/1*a*) and an adenine-independent, canavanine-resistant culture isolated from strain 174/1*d*. There also the segregation was 2 : 2 for adenine independence-dependence, with the results further indicating the loci for adenine requirement and for canavanine-resistance to be genetically independent. The work of Roman (1956), however, shows considerable complexity at the locus for adenine requirement, and the present interpretation that prototrophs isolated from bursts are back-mutants should be taken only as a first approximation.

(e) *The occurrence of bursts in other adenine-requiring strains*

Spontaneously occurring " little " mutants of strain C-34 were isolated, and their respiratory deficiency was verified by Professor B. Ephrussi. Then, as described for standard strain C-34, the " little " strain of adenine-requiring yeast was plated on 0·5 and 5·0 per cent. glucose medium. No bursts were observed in colonies on the 0·5 per cent. glucose medium. Slender, candle-shaped bursts were observed in certain colonies grown on the 5·0 per cent. glucose medium. Cells isolated from these bursts provided prototrophic cultures that formed small, white colonies. The frequency of bursts, considered in terms of the numbers of colonies observed, was considerably smaller than the frequency of bursts in standard strain C-34. The difference in frequencies so calculated may be relatively meaningless, however. Since colony size for " little " strain C-34 is relatively small, the lower frequency of bursts perhaps derives simply from the fact that each colony represents a relatively small population in which spontaneous back-mutation might occur.

Prototrophic cells from bursts of " little " strain C-34 proliferated as bursts when inoculated either into " big " or " little " red colonies of strain C-34 grown on 5·0 per cent. glucose medium. No proliferation into bursts was observed when such cells were transplanted into colonies grown on 0·5 per cent. glucose medium. Under the conditions of the experiments, the factors affecting intracolony selection of prototrophs seem to operate similarly in " big " and in " little " yeast.

A certain amount of preliminary evidence indicates, however, that genotype may affect the intracolony selection of prototrophs. Cells of strain C-34 were treated for 5 minutes with a 0·1 per cent. water solution of beta-propiolactone, which acts as a mutagen against yeast (Srb, 1956). The cells were then plated on yeast extract medium. Of the colonies growing from the small percentage of viable cells, many showed sectors recognisable by variations in colour (fig. 4). Isolation of cells from one such sector provided a culture that was adenine-requiring but whose pigmentation was deep red on standard media and very deep purple on media including 8 per cent. yeast

extract. From other sectors, orange and light pink adenine-requiring strains were isolated. Under appropriate conditions, the purple and the light pink strains produced bursts similar to those produced by strain C-34. The orange strain, however, did not produce bursts, although small white sectors were sometimes observed. Isolations from these sectors gave prototrophic cells. Orange colonies not showing sectors were also proven sometimes to contain prototrophic cells by use of the technique that demonstrated such cells in colonies of strain C-34 grown on 0·5 per cent. glucose. Transplants of prototrophic cells into orange colonies grown on 5·0 per cent. glucose failed to proliferate into bursts. The genetic relationships between the colour variant strains and strain C-34 were not studied.

4. DISCUSSION

The results just described show that bursts of prototrophic cells occur in colonies of red, adenine-requiring yeast under conditions where a partial deficiency of adenine limits growth and where the *p*H of the medium is 4·5. The primary effect of these conditions, with reference to causation of bursts, is the provision of intracolony environments where prototrophic cells proliferate with much greater rapidity than do cells of standard genotype for the colony. This interpretation is forced by the results of experiments where prototrophic cells were actually transferred into colonies of strain C-34 grown on 5·0 and on 0·5 per cent. glucose media. In the former instance (low *p*H), typical bursts invariably developed; in the latter instance (higher *p*H), typical bursts never developed. That a limitation on adenine supply contributes to the burst phenomenon was seen in experiments where successively higher adenine concentration in the medium was paralleled by a reduction in size of bursts until they failed entirely to appear.

None of the results rigidly exclude the possibility that *p*H and adenine concentration may affect the rate of back-mutation at the locus for adenine requirement. However, altered mutation rate seems not to be required for interpretation of the burst phenomenon. A fair proportion of colonies grown on 0·5 per cent. glucose medium, where bursts did not occur, were shown to include varying numbers of prototrophic cells. Since under favourable conditions even a single prototrophic cell appears to have a high probability of expressing itself as a burst, colonies on 0·5 per cent. glucose may be considered to have had the genetic potential for bursts but not to have expressed this potential.

Two aspects of the selection mechanism involved in the burst phenomenon are of particular interest. One of these is the demonstration that very fine differences in environment may have profound effects on the selection of genotypes. Other conditions being appropriate, a difference of 0·5 *p*H units, or perhaps less, determines

whether or not the burst phenomenon may occur. A difference in adenine concentration of only 10 mg. per litre may determine whether incipient bursts develop into bursts. The other striking feature of the results has to do with the intensity of intracolony selection. As noted, results with artificially constructed giant colonies indicate the high probability that a singly occurring prototrophic cell will reproduce as a clone that will escape the normal colony confines, under particular environmental circumstances. In this context, the advantages of the giant colony technique for studying a variety of problems in yeast should be emphasised. Skovsted (1956) has utilised this technique to study mutational phenomena affecting morphology in yeast. The present work illustrates that the technique also has possibilities for studies in selection.

The burst phenomenon has many properties in common with a recurrent developmental event, and may be viewed as such. Colonies that develop bursts show characteristic changes in morphology, become bipartite rather than unipartite in gross structure, and have consistently oriented colour differentiation. With correct adjustment of conditions, almost 100 per cent. of colonies in a series of plates could be made to show bursts. Besides appropriate *p*H and adenine concentration, these conditions included sufficient time, *i.e.* up to 14 days' growth period, and a low frequency of colonies per plate, so that each colony achieved reasonably large size. Under such conditions the phenomenon became highly recurrent indeed.

While the suggestion is not being made here that the basis of differentiation in multicellular organisms is spontaneous somatic mutation, the burst phenomenon provides a model showing that certain kinds of recurrent developmental events might feasibly be based on a mechanism involving spontaneous mutation. In addition the model shows that the operation of such a mechanism need not depend, if selection is sufficiently effective, on an unusually high mutation rate. The frequency of back-mutation in the adenine-requiring yeasts has not been possible to define precisely, but our experiments indicate that it may be somewhere between 1×10^{-6} and 1×10^{-7}. Since yeast colonies grown under the conditions indicated above may contain upwards of $1{\cdot}7 \times 10^{8}$ cells, and since a single back-mutant cell is highly likely to express itself as a burst, the high degree of recurrence of the burst phenomenon becomes comprehensible in terms of standard genetic mechanism.

Finally, the burst phenomenon may have some speculative implications for oncology. Beadle (1945), in discussing the somatic mutation theory of the origin of cancer, points out that " the remarkable thing is not that malignancy changes are as frequent as they are but rather that they are not more frequent ". Failure of intracolony selection of mutant prototrophs at *p*H 5·0 and above, and the consequent absence of bursts, shows that cells which are inordinately proliferative in certain cellular environments may not be so in others. This suggests

Plate

FIG. 1.—Strain 174/1*b* on yeast extract medium with 0·5 per cent. glucose. Colonies are 12 days old, and do not show bursts.

FIG. 2.—Strain 174/1*b* on yeast extract medium with 5·0 per cent. glucose. Colonies are 7 days old, and some show bursts.

FIG. 3.—Colonies of strain C-34 into which prototrophic cells had been introduced after 3 days. Every colony shows a burst. Medium is yeast extract with 5·0 per cent. glucose.

FIG. 4.—Sectored colonies of strain C-34. Cells used for inoculum had been treated with beta-propiolactone.

FIG. 1

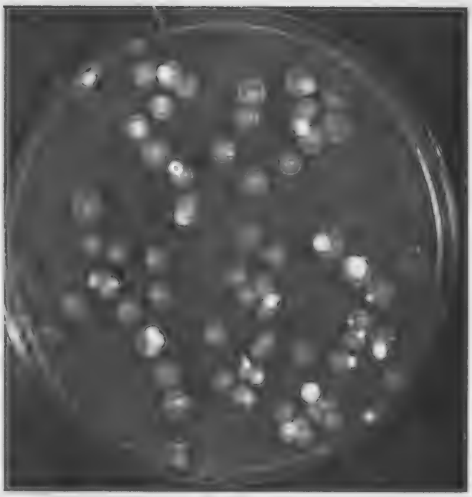

FIG. 2

FIG. 3

FIG. 4

that mutation frequency might be less important than cellular environment in the development of neoplasms.

5. SUMMARY

1. Approximately spherical bursts of white, prototrophic cells frequently arise from colonies of certain strains of red, adenine-requiring yeasts. The occurrence of prototrophs appears to result from back-mutation at the locus for adenine requirement.

2. The bursts occur in colonies grown on media supplemented with 5·0 per cent. glucose but not on media supplemented with 0·5 per cent. glucose. With reference to the burst phenomenon, the primary difference in these media has been shown to be *p*H rather than concentration of carbohydrate. Other experiments have shown that well-developed bursts occur only when adenine is partly limiting growth of the red cells.

3. Prototrophic cells are present in colonies grown under conditions where bursts do not arise. Prototrophic cells transferred into colonies of red yeast always proliferate into bursts if the colonies are grown on media that permit the spontaneous occurrence of bursts; similarly transferred prototrophs never proliferate into bursts if the host colonies are grown on media where spontaneous bursts never occur. The burst phenomenon therefore appears to depend upon environmental conditions such that prototrophs have selective advantages within colonies of adenine-requiring cells.

4. Experiments with artificially constructed giant colonies show that any independently occurring prototrophic cell has a high probability of expressing itself as a burst, when certain environmental conditions are met.

5. Some implications of the burst phenomenon for general problems in development are discussed.

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REVIEWS

A HUNDRED YEARS OF EVOLUTION. By G. S. Carter, M.A., Ph.D. London : Sidgwick and Jackson. 1957. Pp. 206+x. 21s.

THE EVOLUTION OF LIVING THINGS. By H. Graham Cannon, F.R.S. Manchester University Press. 1958. Pp. 180+ix. 12s. 6d.

The centenary of the *Origin of Species* has aroused a number of travellers in the evolutionary train into activity. In some cases it has aroused them from slumber. Not so much Dr Carter who has reviewed the century and gives an account which corresponds with what is widely taught about evolution and Darwinism in university departments almost everywhere. Naturally it is not in touch with recent developments in genetic theory. Naturally its focus is in comparative anatomy and palæontology : to him these are real and tangible things ; the rest is seen at an uncertain distance.

Professor Cannon's contribution is of a different kind. He has slumbered very long. And he seems to have slumbered in the luggage van of the train. For when he rubs his eyes all he finds in focus is the giant figure of Lamarck so long misunderstood.

Professor Cannon's interest in Lamarck has not, however, led him to look at what others have recently said about Lamarck. He is unaware of the scholarly histories, the penetrating experiments and the somewhat novel theoretical issues which have within the last twenty years revised our understanding of Lamarckism. He is also unaware of the displacement of classical genetics by unexpected and paradoxical theories which have slightly revised but greatly strengthened the position of Darwinism.

Let it not be thought that Professor Cannon's work is to be disdained. It is far more exciting than Dr Carter's. It is a book that is worth keeping and those who keep it will be able to bequeath a beautifully produced volume that will be as refreshing for their grandchildren as it has been for them.

C. D. D.

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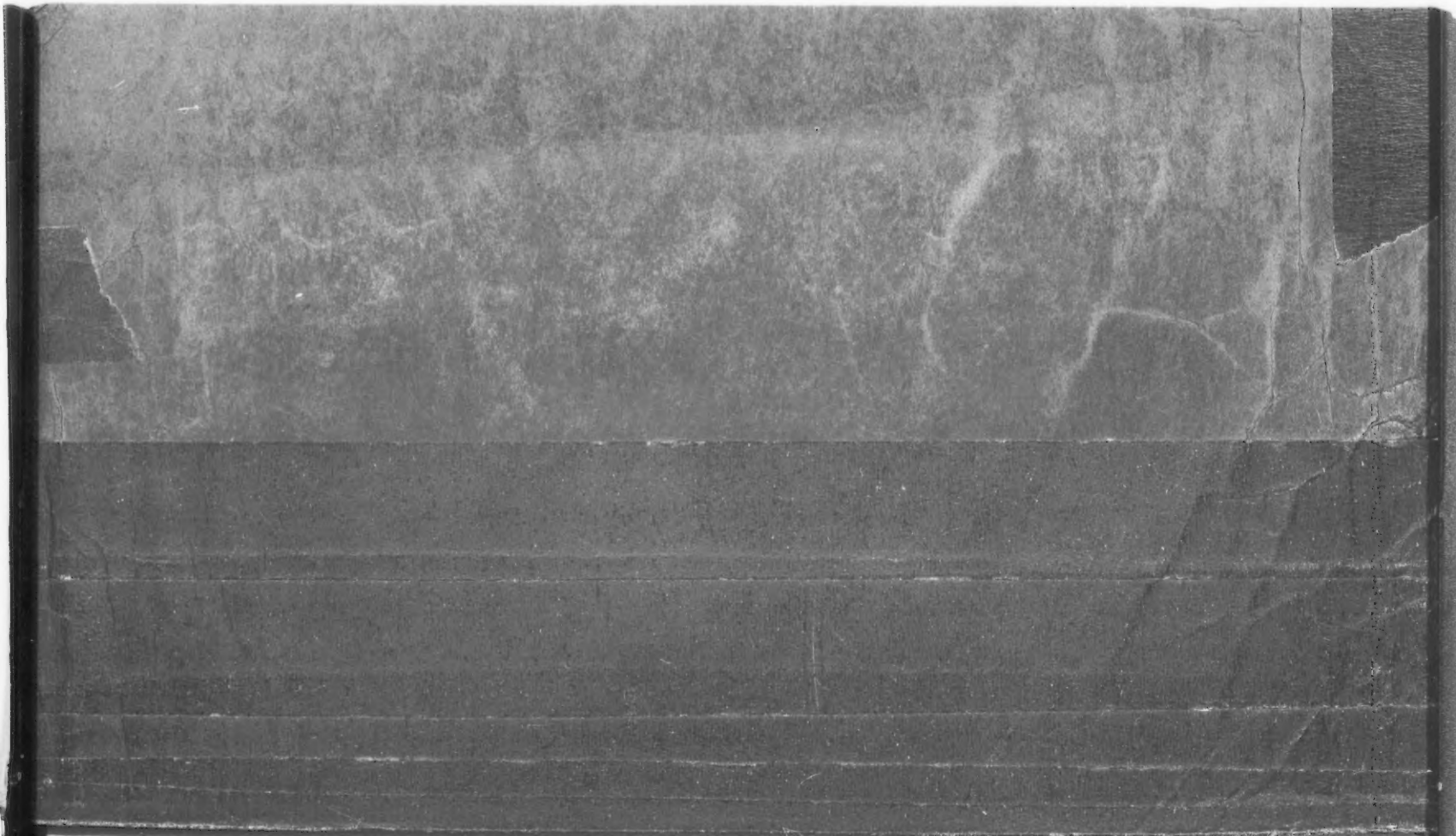
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